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UTILIZATION OF AMINO ACIDS BY SOME FUNGI CAUSING "LEAF SPOT" DISEASE

By

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It is clear from the work of earlier investigators on the physiology of fungi that nitrogen is essential for their nutrition. A review of the literature shows that fungi have definite specificity for the nitrogen compounds they can utilize. A large amount of literature has accumulated on the role of amino acids on the growth and reproduction of fungi and it shows that all amino acids are not of equal value in fungal nutrition. Steinberg (1942) working with *Aspergillus niger* reported that 7 out of 22 amino acids were excellent nitrogen sources for the growth of this organism. According to him these 7 amino acids were primary and from them secondary amino acids were formed. The investigations carried out by Lilly and Leonian (1942) showed that different amino acids have different effect on growth. It also established that different strains of the same organisms may respond differently to the same source of nitrogen.

Many investigators have studied the growth of fungi on mixtures of amino-acids and have tried to compare the growth on individual amino acids with that on their mixture. They have also tried to study the effect of one amino acid on the utilization of the other. Leonian and Lilly (1940) found better growth of *Phycomyces blakesleeanus* on a mixture of 5 amino acids than on the individual amino acids. Lilly and Bernett (1951) reported that a mixture of amino acids may or may not be better utilized than a single amino acid, and that the effect of a single amino acid on the utilization of another varies with the amino acids involved and the fungus concerned. Bilgrami (1956) working with *Pestalotia mangiferae, Phyllosticta cycadina* and *P. artocarpina* found better growth of these organisms on a mixture of 5 amino acids than on individual amino acids. He observed that both poor and good amino acids were assimilated simultaneously from the mixture. Raizada (1957) carried out similar investigations on 7 members of Mucorales and found better growth of all the organisms on a mixture than on individual amino acids. He reported that arginine was preferentially utilized from the mixture by *Mucor hiemalis and M. Rouxii.*

Ram Dayal (1958) working with 4 members of saprolegniales reported that only *Achlya aplanes* made better growth on the mixture of amino acids than on individual acid while rest of the 3 organisms viz. *Isoachlya unispora*, *I. toruloides* and *Saprolegnia parasitica* failed to do so. He observed that *Achlya aplanes* utilized all the amino acids from the mixture almost simultaneously, while the other 3 fungi showed greater choice for arginine as it was consumed earlier than other amino acids. It may, however, be mentioned that this substance was a poor source of nitrogen for the mycelial growth or those three organisms.

In view of the results of various investigators which show that even different strains of the same fungus may respond differently on single amino acid or on their mixture, it was thought desirable to study the utilization of 5 amino acids and their mixture by some fungi causing leaf spot diseases.

MATERIAL AND METHODS

The organisms under investigation, viz., *Cercosporina ricinella* (Sacc. & Berl.) speg., *Colletotrichum gloeosporoides* Penz. and *Curvularia penniseti* Mitra were isolated from infected leaves of *Riccinus communis* Linn., *Punica granatum* Linn. and *Pennisetum typhoideum* Stapf. and Hubb. respectively. The infected leaves were collected from various gardens and farms of the town including the Botanical garden of Allahabad University. Single spore cultures were prepared by dilution method and bacteria were removed by the method described by Brown (1924). Asthana and Hawker's medium A* was selected as the basal medium. The amount of nitrogen in KNO_3 of Asthana and Hawker's medium A (3·5 gms.) was calculated and it was replaced by equal quantity of nitrogen in the following amino acids viz., alanine (Rf. 0·46), glycine (0·37), leucine (0·78), aspartic acid (0·33) and glutamic acid (0·39) and their mixtures. On the basis of previous investigations the pH of the medium was adjusted to 6·5 for *Cercosporina recinella*, 5·9 for *Colletotrichum gloeosporoides* and 5·4 for *Curvularia penniseti*. Pyrex glass wares and purest chemicals were used in the present investigation. Fixed amount of the medium (25 c.c.) was taken in 150 c.c. conical flasks. Four replicates were used for each treatment.

The various media were autoclaved at 15 lbs. pressure for 15 minutes. Solutions containing the amino acids and their mixtures were inoculated with different organisms daily by Garrett's (1936) agar disc method for 15 days and were incubated at a temperature of 25°C (±2°C). The fungal mat from each set was filtred separately on sixteenth day when one to fifteen days old cultures were available. The filterate from each set was chromatographically analysed to detect the presence of amino acid and thus the rate of assimitation was recorded. The growth records were maintained by weighing the fungal growth after every fifth day. The circular paper chromatography technique of Ranjan *et al* (1955) was employed for the analysis of the filterate. A circular piece of Whatman paper No. I having a diameter of 27 cm. (45 cm. for mixture of amino acids) with radial cuts (according to number of days) was used. From the filterate, drops of ·005 ml. were placed at the positions located for this purpose. Equal volumes of drops of known solution were also placed on the same chromatograms to facilitate the identification of the bands. The chromatograms were run with n-butanol-acetic acid-water (4 : 1 : 5) as solvent. The spray reagent consisted of ·1% ninhydrin in normal butanol. The bands were finally developed by placing the chromatograms in the electric oven at a constant temperature of 100°C for about 1 to 2 minutes. The average Rf values of the various amino acids have been calculated on the basis of the bands developed.

* Glucose 5 gms, Potassium nitrate 3·5 gms., Potassium dihydrogen phosphate 1·75 gms., magnesium sulphate 75 gms., distilled water 1000 c.c.

EXPERIMENTAL

The presence of amino acids and their mixture in the medium and dry weight of the 3 organisms on them is given in the Table 1.

TABLE 1

Showing the presence of amino acids and their mixture in the medium and dry weight of *Cercosporina ricinella*, *Colletotrichum gloeosporoides* and *Curvularia penniseti* obtained on them

Amino acids	*Cercosporina ricinella*				*Colletotrichum gloeosporoides*				*Curvularia penniseti*			
	Dry wt. in mgs			Presence of amino acids	*Dry wt. in mgs*			Presence of amino acids	*Dry wt in mgs*			Presence of amino acids
	5 days	10 days	15 days		5 days	10 days	15 days		5 days	10 days	15 days	
Alanine	25·5	43·0	48·0	10 days	56·1	67·1	69·6	7 days	39·8	78·5	94·3	12 days
Glycine	19·0	29·1	33·0	8 ,,	14·0	27·8	31·0	9 ,,	20·3	37·1	42·3	10 ,,
Aspartic Acid	14·5	24·0	36·3	15 ,,	58·3	75·5	81·0	9 ,,	11·0	19·0	22·2	11 ,,
Glutamic Acid	10·7	18·0	24·2	15 ,,	45·1	75·3	90·1	7 ,,	18·0	29·7	43·3	13 ,,
Leucine	14·2	25·1	35·1	15 ,,	20·0	36·2	44·0	12 ,,	19·2	34·1	40·0	10 ,,
Mixture of amino acids	27·1	45·0	54·1		66·0	77·8	84·3		40·5	77·3	89·0	
Alanine				11 ,,				8 ,,				14 ,,
Glycine				9 ,,				5 ,,				10 ,,
Asp. Acid				9 ,,				6 ,,				10 ,,
Glut. Acid				11 ,,				7 ,,				10 ,,
Leucine				11 ,,				8 ,,				14 ,,

Assimilation of glutamic acid (Rf 0·47): It is clear from the Table that out of five amino acids used in the present investigation, this acid was found to be comparatively good source of nitrogen for the mycelial growth of *Colletotrichum gloeosporoides* while it was a poor source for *Cercospornia ricinella* and a moderate source for *Curvularia penniseti*. Chromatographic analysis of the medium showed that *Colletotrichum gloeosporoides* and *Curvularia penniseti* assimilated this acid in 7 and 13 days respectively. *Cercosporina ricinella* was slow in utilizing this amino acid as it could not finish it completely upto 15 days.

Assimilation of aspartic acid (Rf — 0·43). The table shows that aspartic acid could also support good growth of *Colletotrichum gloeosporoides*. The mycelial growth was poor in *Curvularia penniseti* and moderate in *Cercosporina ricinella*. Chromatographic analysis of the medium showed that aspartic acid persisted upto 9 and 11 days in the culture media which were inoculated with *Colletotrichum gloeosporoides* and *Curvularia penniseti* respectively. Even though the growth of *Cercosporina ricinella* was better than that of *Curvularia penniseti* it could not fully assimilate this acid upto 15 days.

Assimilation of alanine (Rf 0·53). Good growth of *Curvularia penniseti* and *Cercosporina ricinella* was obtained on this amino acid but it proved to be only a moderate source of nitrogen for the growth of *Colletotroichum gloeosporides*. Daily analysis of the medium showed that *Cercosporina ricinella* and *Colletotrichum gloeosporoides* could assimilate this acid in 10 and 7 days respectively while *Curvularia penniseti* finished it in 12 days.

Assimilation of glycine (Rf 0·45). This acid supported poor growth of *Colletotrichum gloeosporoides* and moderate growth of *Cercosporina ricinella* and *Curvularia penniseti*. Chromatographic analysis of the medium revealed that it persisted upto 8, 9 and 10 days in media which were inoculated with *Cercosporinia ricinella*, *Colletotrichum gloeosporoides* and *Curvularia penniseti* respectively.

Assimilation of Leucine (Rf 0·80). The dry weight records indicate that this acid was a moderate source of nitrogen for the growth of all the three organisms. Daily analysis of the medium showed that this acid was completely assimilated in 10 and 12 days by *Curvularia penniseti* and *Colletotrichum gloeosporoides* respectively. *Cercosporina ricinella* was slow in utilizing this acid as it could not consume it completely upto 15 days.

Assimilation of mixture of amino acids : Dry weight results show that *Cercosporina ricinella* produced better mycelial growth on the mixture containing all 5 amino acids than on any of them individually. *Curvularia penniseti* and *Colletotrichum gloeosporiodes* differed with this organism as they were able to utilize one or the other amino acid better than their mixture. It was found that *Curvularia penniseti* produced better mycelial growth on alanine while *Colletotrichum gloeosporoides* grew better on glutamic acid than on mixture of amino acids.

Chromatographic analysis of the medium showed that both glycine and aspartic acid were consumed in 9 days by *Cercosporina ricinella* but this organism was slow in utilizing glutamic acid, leucine and alanine from the mixture as they were found to be present in the medium upto 11 days.

Colletotrichum gloeosporoides was able to assimilate glycine from the medium in 5 days only while aspartic acid and glutamatic acid were consumed in 6 and 7 days respectively. Alanine and leucine remained in the medium upto 8 days. Individually each of them was utilized in a longer period.

Curvularia penniseti utilized glutamic acid, glycine, aspartic acid from the mixture in 10 days, while alanine and leucine persisted in the medium upto 14 days.

DISCUSSION

The results obtained clearly showed that the three organisms responded differently for different amino acids. The dry weight of an organism could not always be correlated with the rate assimilation of a particular amino acid, because a fungus may yield satisfactory growth on an amino acid even if the particular amino acid may not be entirely consumed from the medium or *vice versa*. Moderate mycelial growth of *Cercosporina ricinella* was obtained on aspartic acid and leucine but chromatographic analysis of the medium showed that both of these acids were not assimilated from the medium upto 15 days. Glycine suported poor and aspartic acid good mycelial growth of *Colletotrichum gloeosporoides* but irrespective of the growth both the acids were assimilated by the fungus in 9 days. This may be due to difference in availability of different amino acids for the synthesis of cell contents.

It was also evident from the present results that the rate of asssimilation of different amino acids (when supplied individually) had no direct relation with their utilization from the mixture. It is thus evident that the rate of assimilation of amino acids was influenced by the presence of other amino acids in the mixture. An amino acid whose rate of assimilation may be comparatively slower may be preferentially utilized when supplied in a mixture with other amino acids. The reverse has also been found to be correct.

The results further indicated that the suitability of an amino acid (as judged by growth) could not be correlated with their rate of assimilation in a mixture. Any amino acid which might be a poor source of nitrogen for growth of an organism may readily be utilized when given in a mixture or *vice versa.*

Glycine was found to be poor source of nitrogen for the growth of *Colletotrichum gloeosporoides* but when supplied in mixture it was consumed only in 5 days though the other amino acids persisted in the medium for 7—1! days. Alanine supported very good growth of *Curvularia penniseti* but its utilization was very slow from the mixture because it was not consumed from the mixture upto 14 days. *Cercosporina ricinella* gave moderate mycelial growth on glycine but it consumed this acid from the mixture earlier than other amino acids which were found to be better nitrogen sources for growth when supplied individually.

Dry weight results showed that only *Cercosporina ricinella* gave better mycelial growth on the mixture of amino acids than on any individual amino acids. Similar results have been obtained by Bilgrami (1956) for *Plyllosticta cycadina, P. artocarpina* and *Pestalotia mangiferae,* Ram Dayal (1959) for *Achlya aplanes* and Raizada (1957) for 7 members of Mucorales.

Colletotrichum gloeosporoides and *Curvularia penniseti* differed from the above organisms in this respect. *Colletotrichum gloeosporoides* produced better mycelial growth on glutamic acid than on the mixture of all the 5 amino acids together. Similarly *Curvularia penniseti* gave better mycelial growth on Alanine than on the mixture of amino acids.

Ram Dayal (1959) working with *Isoachlya unispora, I. toruloides,* and *Saprolegnia parasitica* found better mycelial growth on histidine, than on a mixture of amino acids.

This different response of different organisms on mixture of amino acids is in accordance with the Statement of Lilly and Barnett (1951 p. 106) in which it has been said that "the effect of one amino acid on the utilization of other varies with the amino acid involved and the organism used."

SUMMARY

The utilization of 5 amino acids and their mixture by three fungi *viz., Cercosporina ricinella, Colletotrichum gloeosporoides* and *Curvularia penniseti,* (isolated from diseased leaves of *Ricinus communis, Punica granatum* and *Pennisetum typhoideum*) was studied. Chromatographic analysis of the medium was also conducted. It was found that *Cercosporina ricinella* was slow in utilizing aspartic acid, glutamic acid and leucine as they were not consumed completely even upto 16th day. It, however, utilised alanine and glycine in 10 and 8 days respectively. Simultaneous utilization of both good and poor amino acids was observed in this case also. This organism gave better mycelial growth on a mixture of amino acids than on any individual form.

Colletotrichum gloeosporoides finished glycine and aspartic acid in 9 days, alanine and glutamic acid in 7 days, while leucine was present in the medium upto 12 days. It was found that this organism could also utilize both good and poor amino acids simultaneously from the mixture. Better mycelial growth of this fungus was found on a mixture of amino acids than on individual ones except on glutamic acid.

Curvularia penniseti could finish leucine and glycine in 10 days. Alanine and aspartic acid in 13 days. Glutamic acid persisted in the medium upto 14 days.

This organism utilized both good and poor amino acids simultaneously from the mixture. It was also found that except on alanine it could give better mycelial growth on a mixture of amino acids than on individual ones.

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HISTOCHEMICAL STUDIES OF THE LIPOIDS IN THE DEVELOPING EGGS OF THE COMMON ENGLISH TROUTS (SALMO TRUTTA AND SALMO IRIDEUS) IV. LIPO-PROTEINS: THEIR LOCATION AND ROLE

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SUMMARY

1. Lipo-proteins in the trout eggs are of many types. Some of them are broken easily, while others with some difficulty.

2. They are spread throughout the egg yolk, and are the first lipoids to be utilised by the embryo.

3. They are selectively used by the embryo. Easily-breakable lipo-proteins are used first.

4. Before the extra-embryonic blastoderm is closed, the lipo-proteins are absorbed without any split. After this event they split into their components to be utilised by the embryo and are reformed inside it. These reformed lipo-proteins are different from those which have been supplied by the yolk.

5. A probable explanation for the formation of the lipo-proteins has been given.

6. A chart of the entire lipoid metabolism has been given at the end.

INTRODUCTION

This is fourth and the last paper of the series. It deals with lipo-proteins. They are formed of a lipoid molecule and a protein molecule linked together with varying strength. According to that they can be dissociated easily or with difficulty. The histochemical reaction of a lipo-protein depends upon the comparative size and probably also the position of the linking molecules. If the lipoid molecule is so placed that it is completely masked by the protein molecule, then it will not be shown by the histochemical techniques, nor will it be shown if the link between the lipoid and protein molecules is so weak that it is broken by simple solvents and the linked lipoids are also dissolved with the free lipoids. Therefore this discrepency does exist in the present study of lipo-proteins. In this paper too, the term 'extra-embryonic blastoderm' has been abbreviated as 'EEB'.

TECHNIQUE

Lipo-proteins remain mixed with other lipoids which react strongly with histochemical techniques. Therefore they can be studied only when others have been removed. This was done by treating the frozen sections of the material fixed in

formaldehyde-calcium with the solvents of triglycerides and those of phospholipines (acetone, cold alcohol and cold ether). The treated sections were stained with sudan black B. Lipo-proteins took a light blue colour (Krishna, 1950). They were also extracted by treating the yolk of trout eggs with M/1 salt solution (Alderton and Fevold, 1945). The treated solution was filtered and the filtrate was dialysed in distilled water for 24 hours. The proteins passed out of the dialyser and the lipo-proteins which were insoluble in water remained inside the dialyser and separated out as an amorphous powder of a light brown colour. Later on the lipo-proteins were treated with lipoid solvents to remove any traces of free lipoids which might have escaped into the salt solution. The lipo-proteins thus obtained did not contain any free lipoids. But certain free proteins could be present there. The details of technique have been published elsewhere (Krishna, 1950).

OBSERVATIONS

General :—The sample of the lipo-proteins obtained in the fashion described above was a mixture of several kinds of lipo-proteins. Some of them were broken easily by hot lipoid solvents or by cooling them to –20°C. These lipo-proteins did not dissolve in lipoid solvents or in water. In some of the lipoid solvents like cold alcohol and cold ether the lipo-proteins did not yield any lipoids. In others (boiling alcohol and boiling ether) the yield was only partial. A very successful process to get the entire amount of lipoids was to freeze the lipo-proteins overnight in ether (McFarlane, 1942). The lipoids were dissolved in ether and the residue did not give any tests for lipoids either by staining with sudan black B or by spot tests. The lipoids dissolved in frozen ether gave positive tests for phospholipines and the residue was positive for organic phosphorus and proteins. Therefore the lipoids taking part in the formation of lipo-proteins were entirely or partly phospholipines and the proteins were phosphoproteins. The formaldehyde fixation had changed the lipo-proteins to the extent that the lipoids did not dissolve out of them so easily as they did before fixation. Boiling alcohol, boiling ether or their mixture had no effect on the frozen sections of the fixed material. The sections withstood the action of benzene and petrol-ether too. Frozen ether also did not completely take out all the sudan-stainable matter. A certain amount was present in the sections the next day. Now, more drastic measures like the use of phosphotungstic, trichloracetic, and nitric acids or incubation with pepsin were required for the complete yield of lipoids. The sections were incubated in 0·06% solution of pepsin (Tiselius and Erikson-Quensel, 1939) in acetic acid at 1·5 pH (Sahyun, 1944).

Lipo-proteins in the yolk :—

When the frozen sections which had been stripped off their triglycerides and phospholipines were stained with sudan black B, there was a light blue colour throughout the section. And with Millon's reagent and sudan black B (Krishna, 1950) the visible colour was uniformly red. Therefore the lipo-proteins were evenly spread all over the yolk region. Whenever a boiling solvent was applied, a certain amount of lipoids was taken out of lipo-proteins, and they became uniformly thin all over the section.

In the beginning of development just below the fertilised ovum there was a thin strip of lipoids containing lipo-proteins. This strip was not so homogenous as the rest of the yolk but was showing droplets of fairly large size. These lipo-proteins were different from those in the rest of the yolk. Even after formaldehyde fixation they could be completely broken by ordinary boiling solvents and yielded the entire

amount of lipoids linked with them ; while those in the rest of the yolk did not yield the entire amount.

The lipo-proteins were the first to come in contact with the embryo and the first to disappear from the yolk. When the yolk droplets in this region came into contact with the embryo, they showed flattening on the sides towards the emdryo and the contents of the droplets collected near their flattened edges. Later on the contents of the droplets decreased and near about the same time the embryo cells that were attached to the flattened droplets contained more lipoids than the other cells. After the EEB is closed lipo-proteins are not observed at the points of contact between the embryo cells and the yolk.

Lipo-proteins in the embryo :—

The embryo lipo-proteins did not dissolve in molar salt solution when fresh embryos were crushed in it. When they were treated with boiling solvents, certain lipo-proteins might have yielded the lipoids. The unbroken lipo-proteins were detected in the embryo residues by histochemical means and by spot tests. When the embryo residues at different stages of development were tested it was seen that the tests for lipoids were negative upto 12 days after fertilisation. Up to 20 days after fertilisation it was some times negative and some times faintly positive. After that period the spot tests for lipoids were always positive. But the sudan black B staining was always negative. The interesting point of this study was that the embryo residues gave negative results for lipoids during the early period of incubation for about a fortnight. Later on the spot tests for lipoids were positive. Obviously there was a certain amount of them so concealed that it was not unmasked by sudan black B, nor was it extracted by the boiling solvents. Further treatment of such residues with other methods like freezing in ether did not extract the entire amount. The spot tests for lipoids were still positive. The negative reactions in the early stages showed that no lipoids were left after the alcohol and ether extractions. If there were any lipo-proteins at this stage they were broken during the process. And the lipo-proteins which had resisted extraction in the advanced embryos had been formed later on.

The positive tests for lipoids became distinct about three weeks after fertilisation. This is the period when muscles become a prominent part of the embroy body and form most of it. Muscle lipo-proteins are known to be a difficult material for lipoid extraction. Halpern (1942) faced difficulty in extracting lipoids from the muscles of salmon roe. Lovern (1948) also found it difficult to extract the entire amount of lipoids from the lipo-proteins of a fish muscle. So was the case with the lipo-proteins of the muscles in trout embryos. It was ascertained by testing the muscles and other parts of embryo separately. About four weeks after fertilisation the trout embryos were about one centimeter long, and more than half of their body on the posterior side was mainly composed of muscles and was very poor in lipoids stainable with sudan black B. On the other hand the anterior part of the body, which was less than half of the embryo body and had very few muscles, was comparatively rich in lipoids stainable with sudan black B. The two parts (anterior and posterior) of fresh embryos were separated with a sharp razor blade, and were seperately tested for lipoids. The results were positive. Later on they were extracted with hot alcohol, hot ether and frozen ether. The residues were tested with sudan black B. The results were negative in both the cases. They were tested for lipoids with spot tests. The results were positive with the residues of the posterior end and negative or faintly positive with the residues of the anterior end. It means that the muscles were not so free from lipoids as they appeared with sudan black B staining.

The lipoids in the muscles were so concealed that they were not reached by sudan black B and could not be extracted by boiling solvents and frozen ether. On the other hand the lipoids in the anterior region of the embryos were stainable with sudan black B and could be easily extracted with boiling solvents.

All these observation show that the lipo-proteins in the embryo were different from those in the yolk. In the early stages of development all the lipo-proteins were broken by boiling solvents. The lipo-proteins which were difficult to break became prominent when the muscles were formed. The muscles did not give any indication their of lipo-protein contents with sudan black B. Although there was every indication that the lipo-proteins in the embryo were different from those in the yolk, their staining properties were similar.

For a few days after fertisation the embryo cells contained only a few lipoid bodies scattered all over. These lipoid bodies contained lipo-proteins which took a light blue colour with sudan black B, and blue with nileblue sulphate. They did not take any stain with sudan IV and red with Millon's reagent. They were completely disorganised by boiling solvents and yielded the entire amount of lipoids contained in them. They were very much like the lipo-proteins of the yolk attached to the embryo at this stage In both the cases the material stainable with sudan black B dissolved entirely in the boiling solvents. And these lipo-proteins differed from those at the centre of the yolk.

At a later stage when the EEB is closed and its endoderm cells are in contact with the yolk, there are lipo-proteins on the surface of the yolk. But they are absent in the lipoid bodies attached to the endoderm cells. The lipoid bodies inside the endoderm cells again showed the presence of lipo-proteins, and these in their properties were different from those on the surface of the yolk. That means that the lipo-proteins of the yolk surface did not pass to the endoderm cells as such. And the lipo-proteins inside the endoderm cells were newly formed. Mesoderm also contained lipo-proreins mixed with other lipoids. But these were different from those in the endoderm cells. And there was no histological or histochemical evidence suggesting any direct relationship between the lipo-proteins in the endoderm cells and those in the mesoderm cells. There were some lipo-proteins in the ectoderm cells also. Again there was no evidence to show any relationship between the lipo-proteins in the ectoderm and those in other layers.

INJECTION EXPERIMENTS

Freshly-extracted lipo-proteins from the yolk of trout eggs were freeze-dried and shaken with ether to remove any free phospholipines. Ether was removed in a current of nitrogen. And the dried lipo-proteins were immediately mixed into triglycerides and injected into the yolk sacs of the larvae (Krishna, 1961). As much quantity of lipo-proteins was mixed as could be easily sucked in the injection needle (no. 18). After freeze-drying, if the lipo-proteins are kept exposed for some time, they do not mix well in triglycerides and increase mortality in the injected larvae. Once they are mixed well in triglycerides, the mixture can be kept for some time without any danger. Some of the larvae were injected with triglycerides only, and some of them were kept uninjected as controls. Also a mixture of triglycerides and proteins (fresh albumen from hen's egg as well as dried egg albumen) was injected into some of the larvae. All the injected as well as uninjected larvae were kept under similar conditions and observed.

RESULTS

When the uninjected larvae had absorbed their yolk sacs, the injected ones had not done so. They were allowed to live for a fortnight more. Triglycerides

without lipo-proteins were not absorbed ; while those with lipo-proteins mixed into them were absorbed in most of the cases. After a week triglycerides with lipo-proteins were completely absorbed. But those without lipo-proteins were as such. The injected lipo-proteins became part of the yolk in a few days and could not longer be differentiated from others already present there. It was clearly indicated that the lipo-proteins helped the absorption of triglycerides and had served the purpose of phospholipines. The mixture of triglycerides and fresh hen's egg albumen proved fatal to all the injected cases, and the larvae died within twelve hours. But the mixture with dried hen's albumen did not produce any adverse effect, although the triglycerides were not absorbed. This indicates that proteins were not the factor to help absorption of triglycerides in the injected cases.

DISCUSSION

The observations indicate that the lipo-proteins are spread throughout the yolk. They are of many kinds. Some of them yield lipoids easily while others do so with some difficulty. Their lipoid portion consists mainly or entirely of phospholipines. Lipo-proteins are the first lipoids to be used by the embryo. In the beginning of development they are absorbed as such ; and they are those which yield lipoids easily. This suggests a selective utlisation of the lipo-proteins by the embryo or their selective supply by the yolk.

Later on when the EEB is formed lipo-proteins come near the embryo, break into their components and phospholipines are set free. At this stage lipo-proteins are absent in the lipoids droplets on the EEB. But they are present in the embryo cells. If the entire passage of the lipo-proteins from yolk to the embryo is considered together, the lipo-proteins are absent at the intermediate stage ; and they are seen again inside the embryo cells. It appears that the lipo-proteins of the yolk are broken at its surface and are reformed inside the embryo. Reformation of the lipo-proteins has further evidence that they are different in the embryo from those in the yolk. The lipo-proteins in the embryo muscles do not stain with sudan black B and are very difficult to break. Such lipo-proteins are not found in the yolk. The breaking of lipo-proteins was also indicated in the injection experiments. Lipo-proteins had helped the absorption of triglycerides and had served the purpese of phospholipines (Krishna, 1961). Triglycerides injected without lipo-proteins were not absorbed. Also those injected with proteins were not absorbed too.

Summing up the entire process of utilisation of lipo-proteins, in the beginning of development they find their way into the embryo as unbroken compounds. Later on they are broken on the surface of the yolk and phospholipines are set free. Lipo-proteins are reformed inside the embryo. Further investigations may show the purpose of such a phenomenon, whether the formation of lipo-proteins is a device to economise space occupied by phospholipines and the energy contained in them, so that the organism can make use of the hidden phospholipines whenever needed by breaking the complex molecule.

Since this is the last paper of the series, it would not be out of place to sum up the results of the previous papers also to have a comprehensive picture of the lipoid metabolism in the development of trout. To avoid repetition I propose to give here only a chart representing the entire lipoid metabolism as found by histochemical means during the course of this study (Fig. 1.).

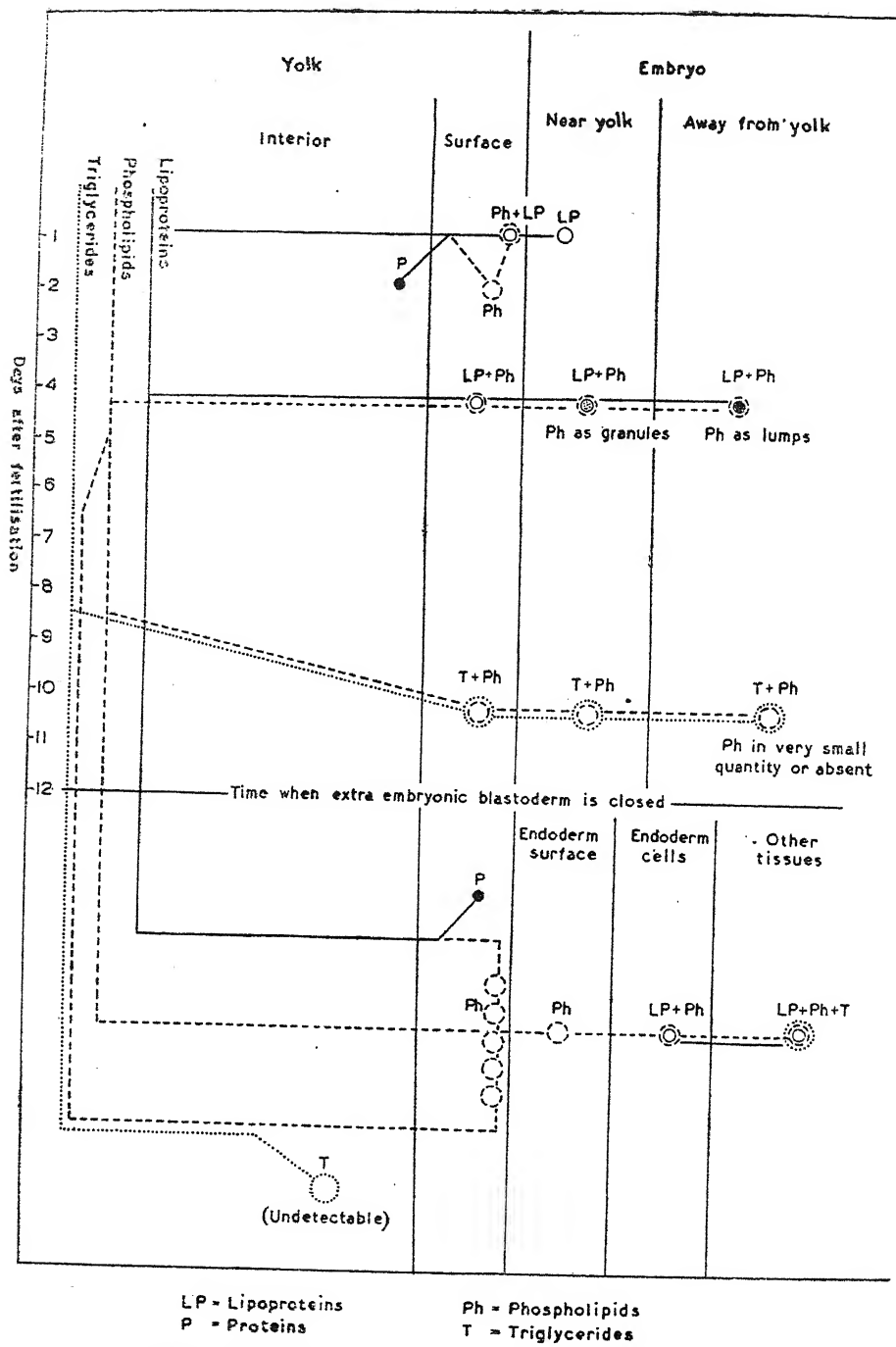

Fig. 1. A diagramatic representation of the entire lipoid metabolism during the incubation period of trout.

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NUTRITIONAL REQUIREMENTS OF *STREPTOMYCES GRISEUS* AGRA STRAIN ON THE PRODUCTION OF A FUNGISTATIC SUBSTANCE. I. CARBON SOURCES

By

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INTRODUCTION

The author reported (1959) a new strain of *Streptomyces griseus* identified as Agra strain and found it antagonistic to *Alternaria solani* and a few other fungi and bacteria. Actinomycetes are able to utilize a great variety of simple and complex organic compounds as Carbon sources (Münter 1916, Pridham and Gottlieb 1948, Hirch 1951, Hirch and Wallace 1951, Perlman and Wagman 1952). Waksman *et al* (1946) observed that the carbon sources as nutrients are not specific for the production of the antibiotic produced by *Streptomyces griseus* and observed that starch and glycerol can be substituted for glucose in the basal medium. But Dulaney and Perlman (1947), Dulaney (1949), Hubbard and Thornberry (1947) observed that streptomycin production is low or absent except when glucose, starch or maltose is present. Hubbard and Thornberry (1947) further observed that *Streptomyces griseus* can utilize d-mannose, d-galactose, cellobiose, maltose, mannitol and dextrin both for the production of streptomycin as well as its growth but it cannot use l-arabinose, l-xylose, rhamnose, lactose, melibiose, trehalose, raffinose, melizitose, dulicitol, inulin, glycogen or gum arabica. This investigation was taken up with the view to find out whether the carbon sources are specific in the production of the fungistatic substance by the Agra strain of *Streptomyces griseus* and under what conditions of carbon nutrients the production is maximum.

MATERIAL AND METHOD

Streptomyces griseus Agra strain was cultured in a basal medium composed of asparagine 0·5 gm., dipotassium phosphate 0·5 gm., and magnesium sulphate 0·25 gm., per litre of distilled water. Carbon sources such as xylose, galactose, arabinose, glucose, sucrose, maltose, fructose, lactose, glycerol and mannitol were added to the medium at the rate of 8% of carbon. The initial pH was adjusted at 7·0 before autoclaving. 30 ml. of the medium was distributed to flat bottles and autoclaved at 10 lbs. pressure for half an hour and then inoculated. The bottles were incubated at about 25°C in an incubator and the fungistatic activity was measured at regular intervals of 7, 10, 13 and 16 days after which the mycelial dry weight was obtained and the final pH of the basal medium determined.

Because glucose was found to be most suitable of all carbon sources, its effect was studied in greater detail.

RESULTS

TABLE I

Effect of carbon sources on the growth of *Streptomyces griseus* Agra strain and the production of the fungistatic substance in terms of 'SD' units. (mean of 5 readings).

Sources	D 7	A 10	Y 13	S 16	Initial pH	Final pH	Dry weight in gms.
Xylose	4·0	8·0	16·0	32·0	7·0	8·8	0·8331
Arabinose	3·2	7·2	16·0	32·0	7·0	5·2	0·8329
Glucose	32·0	64·0	128·0	256·0	7·0	7·3	1·6895
Sucrose	12·8	25·8	51·1	64·0	7·0	8·8	1·0341
Galactose	8·0	28·8	50·8	113·6	7·0	8·2	1·3827
Fructose	16·0	32·0	64·0	128·0	7·0	8·4	1·4015
Maltose	14·2	32·0	64·0	128·0	7·0	7·9	1·4015
Lactose	25·6	44·8	64·0	128·0	7·0	7·6	1·4020
Glycerol	4·0	6·4	8·0	16·0	7·0	4·0	0·4702
Mannitol	0·0	2·4	4·0	8·0	7·0	4·0	0·0152

TABLE II

Showing the effect of glucose concentrations on the growth of *Streptomyces griseus* Agra strain and the production of the fungistatic substance in terms of 'SD' units. (mean of 5 readings).

Concentration	D 7	A 10	Y 13	S 16	Initial pH	Final pH	Dry weight in gms.
2%	2·4	4·8	8·0	16·0	7·0	7·6	0·4691
4%	8·0	16·0	32·0	51·2	7·0	7·9	0·9994
6%	22·4	38·4	89·6	179·2	7·0	7·9	1·4040
8%	32·0	64·0	128·0	256·0	7·0	7·3	1·6881
10%	16·0	23·0	64·0	128·0	7·0	7·3	1·3990
12%	8·0	8·0	16·0	32·0	7·0	6·7	0·9213
14%	4·0	8·0	16·0	32·0	7·0	5·5	0·8923

The results presented in Table I indicate that *Streptomyces griseus* Agra strain can utilize in varying degrees all the carbon sources used in the experiment. The effect of these sources is similar both on the growth (dry mycelial weight) and the production of the fungistatic substance showing thereby a direct relationship between the two. Of all the carbon sources glucose supports the best growth (1·6895 gm., mean dry mycelial weight) and also gives the highest production of the fungistatic substance (256 'SD' units). Glucose is followed in the descending order

by lactose, maltose, fructose, galactose, sucrose, xylose and arabinose. Glycerol and mannitol give the poorest yield of the active substance.

In most cases the pH of the basal medium changed towards alkalinity except in arabinose, glycerol and mannitol where the change was for acidity. At pH 7·1–7·4 the growth and the production is the best whereas at pH 8·4 the production goes down slightly. On lower pH values the production is poor.

Because glucose was found to be the best source of utilization of carbon, the influence of varying amounts of this sugar was studied in detail. The results are tabulated in Table II. The figures indicate that as the glucose concentration is increased the production and also the dry weight increases and the highest values for both are obtained in 8% glucose concentration. In still higher concentration the production as well as the dry weight come down. The final pH level of the basal medium also appears to be related to glucose concentration. On lower concentrations the final pH tends towards alkalinity while the pH falls to acidic side if 12% or 14% of glucose concentration is used.

DISCUSSION

The results of the experiment are in conformity with the earlier observations that Actinomycetes generally can utilize a number of sugars, sugar alcohols etc. for their metabolism. The present observations, however, contradict the view of Waksman *et al* (1946) that carbon is non specific in the production of streptomycin, for glucose can be effectively replaced by starch or glycerol. In the present investigation all the ten carbon sources have not behaved alike with respect to production of the fungistatic substance and growth of the organism. Glucose gives the highest titre value with 256 'SD' units and mannitol the lowest with 8 'SD' units. In between these two extremes the remaining carbon sources can be placed either singly or in groups *viz.* fructose, maltose and lactose each with 128 'SD' units, galactose with 113·6 'SD' units, sucrose with 64 'SD' units, xylose and arabinose with 32 'SD' units and glycerol with 16 'SD' units. The grouping of carbon sources hold good in respect to the growth of the organism as well. This shows the specificity of carbon source for the production of active substance by *Streptomyces griseus* Agra strain. This strain does not utilize glycerol whereas in other cases the organism utilized the same readily. Waksman *et al* (1946) could substitute glycerol for glucose for good production of streptomycin. *Streptomyces griseus* Agra strain is unable to utilize mannitol as observed by Hubbard and Thornberry (1947) in case of other strains of *Streptomyces griseus*. In the present experiment it is found that sugars are better utilized than alcohols.

The carbon requirements also differs from species to species and even in the same species different strains have different carbon requirments for their growth and the production of antibiotic substances. Brian *et al* (1946) found that maltose was unsuitable for strain 10 and 213 of pigment forming strain of *Trichoderma viride* but it was very good for strain 214 of the same species. Similarly lactose was observed to be effective for strains 10 and 214 and not for strain 213. The carbon requirement of *Streptomyces griseus* Agra strain differs from that of the other species of *Streptomyces* as given by Pridham and Gottlieb (1948).

In the case of production of gladiolic acid and alternaric acid (Brian *et al* 1948, 1951) the production increases with the increase in glucose concentration but in the present investigation with the increase in glucose concentration from 2% to 8%, the production of the fungistatic substance also increased. On further increase

in glucose concentration, the production of the fungistatic substance decreased gradually. This fall may be due to the fact that excess glucose (more than 8%) was not utilized and it formed lactic acid which hinders the formation of the active substance. Lactic acid has been detected as a metabolic product in the medium.

SUMMARY

This paper deals with the effect of ten different carbon sources *viz.* xylose, arabinose, glucose, sucrose, maltose, fructose, lactose, galactose, glycerol and mannitol on the production of a fungistatic substance produced by *Streptomyces griseus* Agra strain which is a new strain.

All the carbon sources tested were utilized but to varying degrees. Glucose was the best source yielding 256 'SD' units of fungistatic substance at 8% concentration followed in the descending order by lactose, maltose, fructose, galactose sucrose, xylose, arabinose, glycerol and mannitol with 128, 128, 128, 113·6, 64, 32, 32, 16 and 8 'SD' units respectively.

The production of the active substance and growth of the organism increased as glucose concentration was raised from 2% to 8% above which there was gradual fall in the fungistatic activity as well as the mycelial dry weight.

ACKNOWLEDGMENTS

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*Original not seen.

A STUDY OF THE ATRETIC FOLLICLES IN THE OVARY OF THE DOMESTIC PIGEON*

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INTRODUCTION

Henneguy (1894), Dubuisson (1905 and 1906), Loyez (1906), Stieve (1918*a*, *b*) and Hett (1923) are some of the earlier workers who made observations on the follicular atresia in birds. Pearl and Boring (1918) compared the atretic follicle in the fowl to the mammalian corpus luteum ; this homology is denied by Fell (1925). Rowan (1930), Wynne-Edwards (1939), Davis (1940, 1942 and 1944) and Dunham and Riddle (1942) noticed the bursting type of atresia in several species of birds. Bullough (1942), Fraps and Dury (1942), Rothchild and Fraps (1945), Moreau, Wilk and Rowan (1947), Bretschneider and De Wit (1947), Marshall and Coombs (1957) and Dominic (1960*a*, *b*, *c*,) are the more recent workers on the problem. Brambell (1956) has reviewed most of the literature bearing on follicular atresia of vertebrates.

MATERIAL AND METHODS

Ovaries of about 150 domestic pigeons, *Columba livia* Gmelin, all round the year were used in this study. Birds were killed by decapitation, and the ovary quickly removed and fixed in fluids like Bouin's Allen's PFA[3], Allen's B-15, Zenker's, Mercuric-formol, Susa and Corrosive-acetic. Tissues were embedded in paraffin, sectioned at 5 or 7½ μ and stained mostly in Heidenhain's iron haematoxylin and eosin or Mallory's triple stain. Delafield's haematoxylin and Heidenhain's Azan stain were also used. Material fixed in Corrosive-acetic was stained in bulk with Borax carmine and counterstained with Picro-indigo-carmine or Picronigrosin.

OBSERVATIONS

Classification of the Atretic Follicles in the Domestic Pigeon :

Atretic follicles can be classified into three main groups :

I. Bursting atresia or atresia by rupture of the follicular wall. This is further divisible into :—

   A. Those in which the ovular contents flow out into the stroma of the ovary, the mesothelium of the ovary not rupturing. This is subdivided into (a) bursting atresia of heavily-yolked mature eggs, (b) of immature eggs and (c) of eggs with little or not yolk.

   B. Those in which the mesothelium of the ovary breaks and the ovular contents escape into the body cavity.

*This paper formed a part of a thesis approved for the degree of Doctor of Philosophy of the Banaras Hindu University in 1959.

II. Follicular atresia in which the contents are resorbed *in situ*. This is divided into :—

A. *In situ* atresia of heavily-yolked eggs.

B. *In situ* atresia of immature eggs.

C. *In situ* atresia of eggs with little or no yolk.

III. Cystic degeneration which may be of two kinds.

These three types (I, II, and III) of follicular atresia (vida supra) will be dealt with separately in detail.

I. BURSTING ATRESIA

I-A (a) *Bursting atresia of heavily-yolked eggs.*

This type of atresia affects follicles which measure more than 1600 μ in diameter and can be said to occur in six stages (Pl. I, Figs. 1– 6).

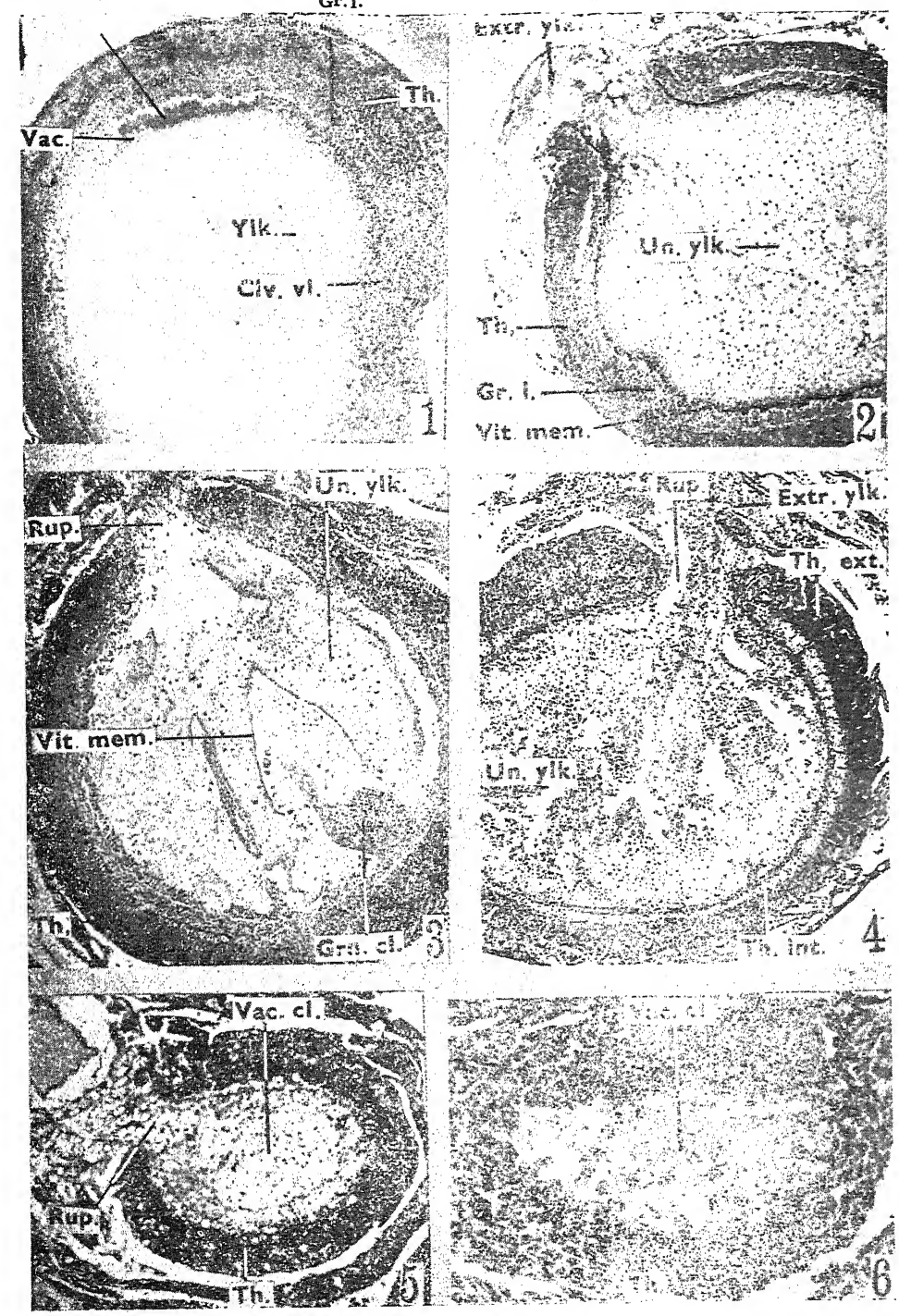
Stage I.—(Pl. I, Fig. 1) The beginning of atresia is marked by the thickening of the vitelline membrane and the appearance of vacuoles in the yolk just beneath it. The germinal vesicle stains feebly and the nuclear membrane tends to disappear. The granulosa cells hypertrophy and clavate villi from the granulosa layer project into the yolk and, therefore, the vitelline membrane becomes tucked in at many places. The membrane propria is present as a very thin layer. The theca interna consists of an inner cellular layer and an outer fibrous layer. The theca externa is fibrous. Both the thecal layears are well vascularised.

Stage II (Pl. I, Fig. 2) is characterised by the dissolution of the germinal vesicle and the rupture of the theca and the extrusion of the liquefied yolk into the spaces in the ovarian stroma. The vitelline membrane greately thickens and invaginates deep into the yolk at certain places carrying with it many granulosa cells. The membrana propria fades away and finally disappears. At one place adjacent to the theca interna, the granulosa cells dissolve away forming a clear space. The vitelline membrane together with the liquefied yolk invades this space ; then the adjoining region of the theca ruptures and the follicular contents flow out into the sinuses in the stroma of the ovary. The rupture may occur at any point on the theca and it may be also at more than one place.

Stage III (Pl. I, Fig. 3) is characterised by the fragmentation of the vitelline membrane, parts of which may be extruded into the stroma. The bulk of the liquefied yolk and some granulosa cells escape into the stroma. Erythrocytes are met with in the follicular lumen. A narrow layer of connective tissue appears along the inner margin of the theca interna.

Active phagocytosis of the ovular debris is an important feature of this stage. The phagocytes are derived from the granulosa cells, and they engulf the yolk and digest them ; undigested yolk particles may also be detected in their cytoplasm. Occasionally phagocytes are seen to arise from the wall of the sinusoids in the stroma and, in all probability they are modified stromal cells. The unextruded yolk that remain in the follicular lumen is eliminated by the granulosa cells that are left behind.

Stage IV (Pl. I, Fig. 4).—The follicle is shrunk to less than half of its original size and most of the extruded yolk globules are already eliminated by phagocytosis. Some unresorbed yolk may persist in the follicular lumen. The inner connective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Vit. mem.
2
Un. ylk.
Rup.
Vit. mem.
Th.
Grn. cl.
3
Rup.
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4
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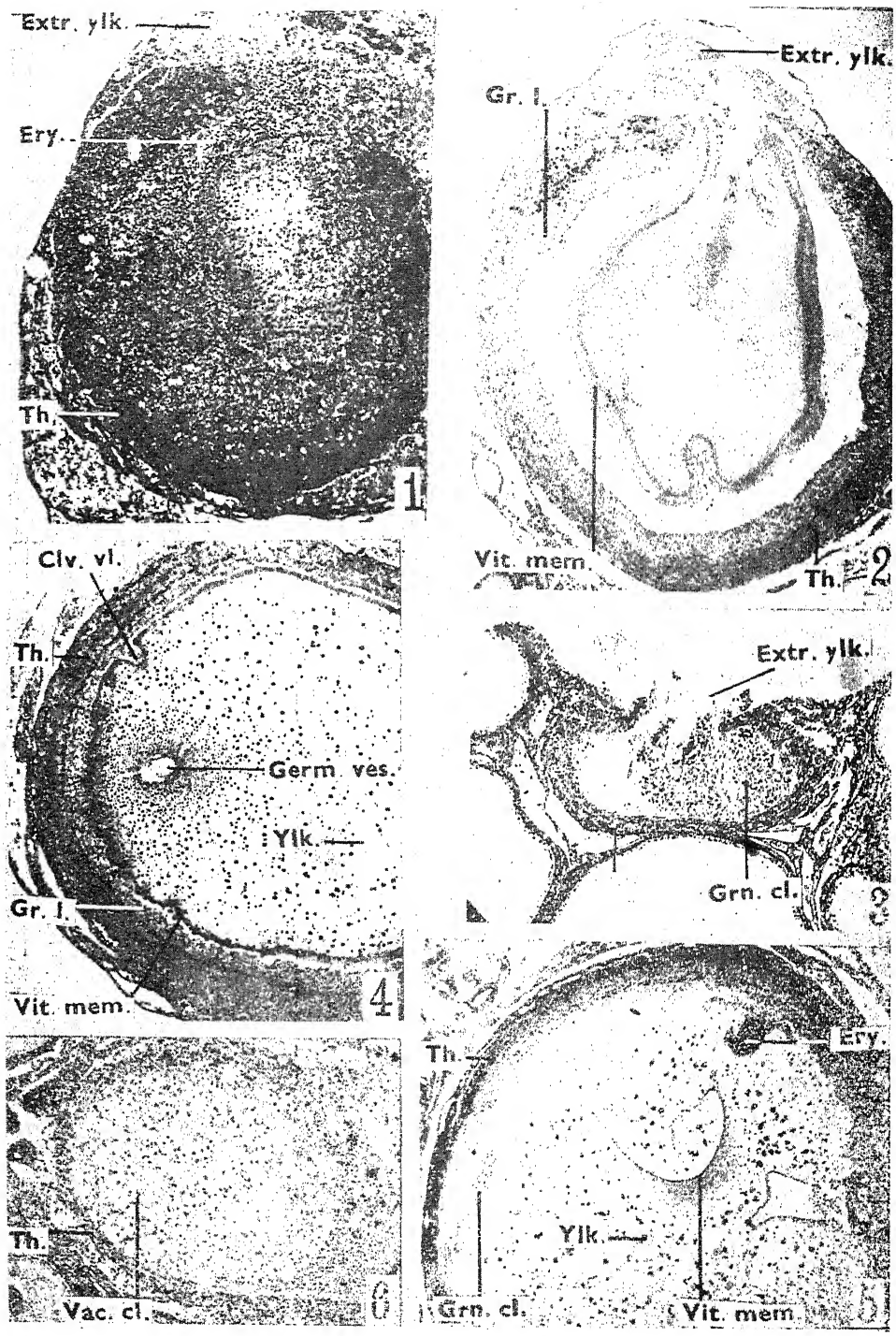
Rup.
Th.
5
6

PLATE I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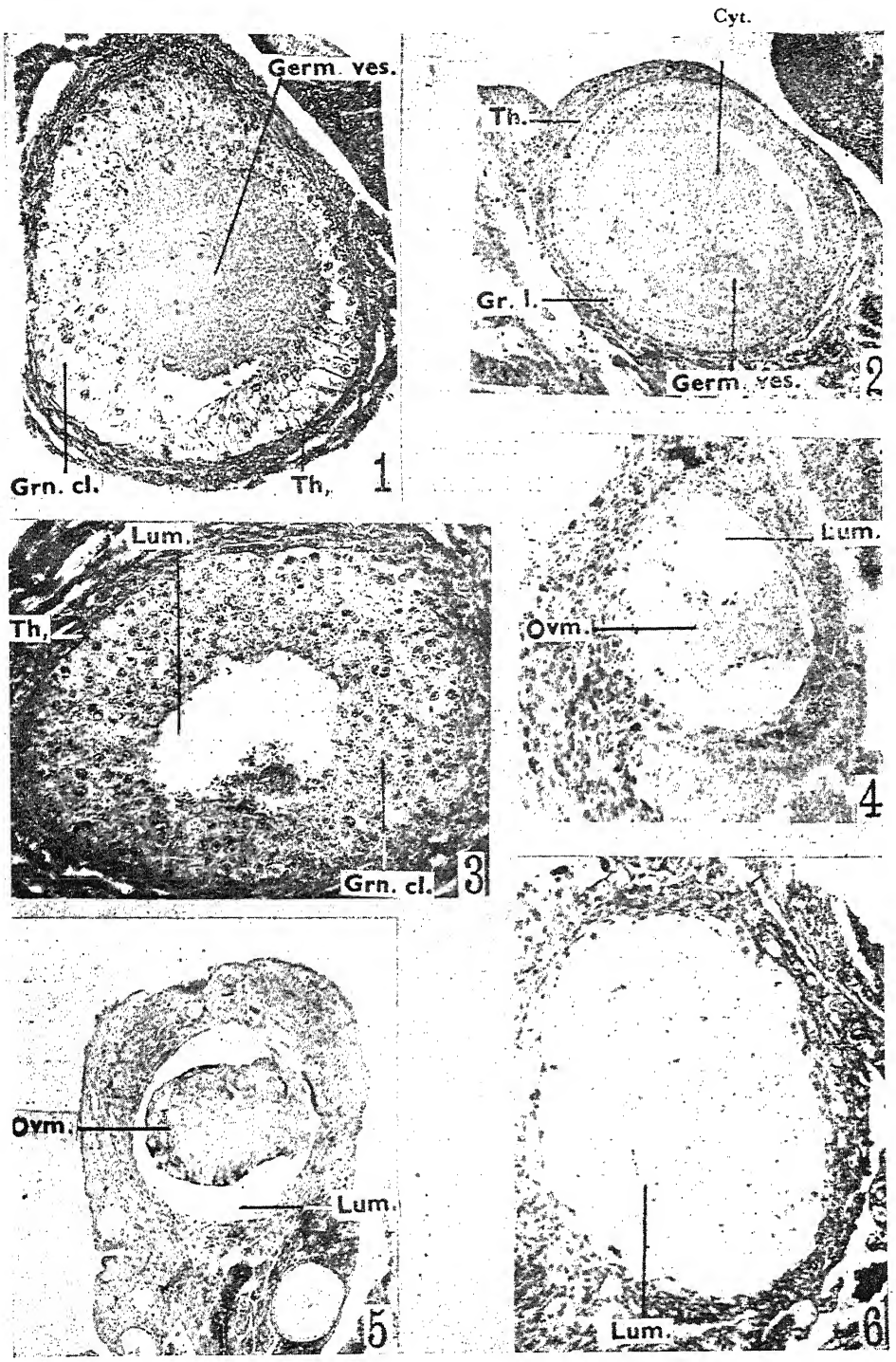
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Th.
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Th.
Gr. l.
Germ. ves.
2
Lum.
Th.
Grn. cl.
3
Lum.
Ovm.
4
Ovm.
Lum.
5
Lum.
6

PLATE III

EXPLANATION OF PLATES

PLATE—I

Fig. 1. Photomicrograph of stage I of the bursting atresia of a heavily yolked egg showing the hypertrophied granulosa layer, thickened vitelline membrane and vacuoles underneath the vitelline memrbane. X 40

Fig. 2. Photomicrograph of stage II of the bursting atresia of a heavily yolked egg showing the dissolution of the yolk, the rupture of the theca and the extrusion of the yolk into the stroma. X 40

Fig. 3. Photomicrograph of stage III of the bursting atresia of heavily yolked egg showing the fragmentation of the vitelline membrane and the extrusion of the ovular debris into the stroma. X 40

Fig. 4. Photomicrograph of stage IV of the bursting atresia of a heavily yolked egg showing the thickened theca, the inner connective tissue layer of the theca interna and the extrusion of the ovular debris into the stroma. X 40

Fig. 5. Photomicrograph of stage V of the bursting atresia of a heavily yolked egg showing the thickened theca and the mass of vacuolated cells in the lumen. X 100

Fig. 6. Photomicrograph of stage VI of the bursting atresia of a heavily yolked egg showing the closure of the ruptured area, the thickened theca and the vacuolated cells in the lumen. X 480

PLATE—II

Fig. 1. Photomicrograph of a burst blood follicle showing erythocytes in the follicular lumen and in the stroma. X 50

Fig. 2. Photomicrograph of the bursting atresia of an immature follicle showing the ruptured theca, the extrusion of the yolk into the stroma and the separation of the vitelline membrane from the granulosa. X 100

Fig. 3. Photomicrograph of the bursting atresia of a follicle showing the extrusion of the ovular content into the body cavity. X 30

Fig. 4. Photomicrograph of stage I of *in situ* atersia of a heavily yolked follicle showing the hypertrophied granulosa layer, thickened vitelline membrane and the distintegrating germinal vesicle. X 40

Fig. 5. Photomicrograph of stage II of *in situ* atresia of a heavily yolked follicle showing the loose granulosa layer, haemorrhage of thecal capillaries, fragmentation of the vitelline membrane and liquefatian of the yolk. X 40

Fig. 6. Photomicrograph of stage IV of *in situ* atresia of heavily yolked follicle showing the thin theca and the vacuolated phagocytes in the lumen. X 200

PLATE—III

Fig. 1. Photomicrograph of a later stage in the *in situ* atresia of an immature follicle showing the hypertrophied granulosa layer, cytoplasm and the germinal vesicle. X 240

Fig. 2. Photomicrograph of *in situ* atresia of an immature follicle in which the cytoplasm adjacent to the granulosa layer dissolved away, showing the germinal vesicle in it. X 100

Fig. 3. Photomicrograph of a later stage of atresia than that shown in Fig. 2 to show the hypertrophied granulosa cells and a central empty lumen. X 200

Fig. 4. Photomicrograph of the *in situ* atresia of an oogonium showing a shrunken ovum in the follicular cavity. X 460

Fig. 5. Photomicrograph of a follicle undergoing cystic degeneration showing a fibroid ovum contained in the follicular cavity. X 100

Fig. 6. Photomicrograph of simple cystic degeneration of a follicle showing a clear central cavity lined by the theca. X 240

KEY TO LETTERING OF FIGURES

Clv. vl.	...	Clavate villi	Rup.	...	Point of rupture of theca
Cyt.	...	Cytoplasm	Th.	...	Theca
Ery.	...	Erythrocytes	Th. ext.	...	Theca externa
Extr. ylk.	...	Extruded yolk	Th. int.	...	Theca interna
Germ. ves.	...	Germinal vesicle	Un. ylk.	...	Unextruded yolk
Grn. cl.	...	Granulosa cell	Vac.	...	Vacuoles
Gr. l.	...	Granulosa layer	Vac. cl.	...	Vacuolated cells
Lum.	...	Lumen	Vit. mem.	...	Vitelline membrane
Ovm.	...	Ovum	Ylk.	...	Yolk

tissue layer of the theca interna becomes very prominent and the theca interna cells have a vacuolated appearance. The theca externa becomes very fibrous.

Stage V (Pl. I, Fig. 5).—The follicle becomes reduced to less than one-fourth of its original size. All the extruded yolk globules are already eliminated by phagocytes and the phagocytes, in their turn, degenerate and disappear. The follicular lumen is filled with a mass of vacuolated cells which are derived from the granulosa. The theca becomes very fibrous and the two layers are not distinguishable.

Stage VI (Pl. I, Fig. 6).—The final stage closely resembles the advanced stages of other types of atretic follicles. The ruptured area is closed by the growth of the thecal walls. The connective tissue in the theca gradullay disappears and the atretic follicle is now represented by a core of vaculated cells in the ovarian stroma. Finally the surrounding stroma invades the follicle and it is thus obliterated. Occasionally hyaline areas resembling the corpus albicans of mammals are left behind by the atretic follicles in the ovarian stroma.

Blood follicles (Pl. II, Fig. 1) sometimes result during bursting atresia of mature follicles. These blood follicles are formed by the excessive haemorrhage of thecal capillaries when the follicular lumen becomes filled with blood. A part of it escapes out along with the yolk globules, but much of it remains in the follicular cavity. The blood islands undergo pathological changes and persist long enough to impart a dark hue to the sections stained with iron haematoxylin (Dominic, 1960*c*).

I. A. (b) *Bursting atresia of immature follicles* (Pl. II, Fig. 2)

Here the vitelline membrane thickens and with the yolk shrinks away from the granulosa layer, then the thecal wall ruptures and the ovular debris flows out. The granulosa layer hypertrophies, and is in tact adhering to the theca interna. Later changes are similar to what takes place in the case of the bursting atresia of heavily yolked follicles.

I. A. (c) *Bursting atresia of follicles with little or no yolk.*

These follicles measure less than 125 μ in diameter. The granulosa hypertrophies and encroaches into the cytoplasm. The theca ruptures and the cytoplasm escapes out, and later the aperture is closed by the growth of the theca. This type of atresia is not frequent and occurs mostly in ovaries during the dormant part of the annual cycle.

I. B. *Bursting atresia in which the ovular contents are extruded into the body cavity* (Pl. II, Fig. 3).

Here the mesothelium of the ovary breaks and the thecal wall ruptures and the yolk escapes out into the body cavity instead of wending its way into the stroma of the ovary. This type of atresia is rather rare as it has been observed only in a few cases (Dominic, 1960, *a*).

II. ATRESIA *IN SITU*

Here the disintegrating ovum is resorbed *in situ*. There is no rupture of the thecal wall and the consequent extrusion of the yolk. This type of atresia shows three variants.

II. A. *In situ atresia of heavily-yolked eggs.*

These follicles measure more than 2000 μ in diameter and the changes taking place during atresia can be described in four steps:

Stage I (Pl. II, Fig. 4).—The preliminary changes are similar to those described in the case of the bursting atresia of heavily-yolked eggs. The vitelline membrane thickens and intrudes into the yolk at many places. Sometimes the invaginations may be so deep into the yolk as to drag the adjacent granulosa cells also. The granulosa cells hypertrophy and produce clavate villi into the yolk.

Stage II (Pl. II, Fig. 5).—The dissolution of the yolk begins and the vitelline membrane fragments and lies scattered in the follicle. The granulosa cells become detached and appear scattered throughout the follicular cavity ; they turn phagocytic and engulf the yolk particles. Erythrocytes resulting from the haemorrhage of thecal capillaries may also be seen in the follicular lumen. The follicle contracts in size and hence the theca becomes more fibrous.

Stage III.—The follicle is now reduced to less than half of its original size. The granulosa cells completely eliminate the yolk by ingestion and then become vacuolated and fill the follicular lumen. The theca interna cells also become vacuolated ; the thecal layers become extremely fibrous.

Stage IV (Pl. II, Fig. 6) bears a strong resemblence to the final stages of other types of atresia. The follicle further contracts, the connective tissue of the theca first becomes prominent and then gradually disappears. The granulosa phagocytes become pycnotic and are eliminated. Finally all that is left of the follicle is a clear area containing some vacuolated cells in the stroma of the ovary. This is gradullay obliterated by the invasion of the fibrous tissue from the ovarian stroma.

II. B. *In situ atresia of immature follicles* (Pl. III, Fig. 1).

These follicles do not measure more than 400 μ in diameter. The peripheral cytoplasm shows signs of disolution and the granulosa cells hypertrophy and begin to ingest the yolk-laden cytoplasm from the periphery. The germinal vesicle remains intact. As atresia progresses, the ovular cytoplasm becomes gradually lost and the granulosa cells reach the germinal vesicle which is later phagocytised and a mass of vacuolated cells is left in the lumen. Gradually the surrounding connective tissue grows in and the atretic follicle is lost in the stroma of the ovary.

Departures from the above type are met with in certain cases :

(*i*) In some follicles the granulosa cells hypertrophy to form a two or three cell thick layer. Then the yolk-laden cytoplasm dissolves close to the granulosa layer and a clear space is formed between the two (Pl. III, Fig. 2). As atresia progresses the cytoplasm dissolves more and more, until the total contents disappear, leaving behind a spaceous cavity lined by the granulosa cells (Pl. III, Fig. 3).

(*ii*) In some follicles the process of destruction is carried out by two agencies, *viz.*, the granulosa cells and the leucocytes. The granulosa cells hypertrophy and destroy the peripheral cytoplasm ; along with this, leucocytes appear in the cytoplasm throughout the follicle.

II. C. *In situ atresia of follicles with little yolk* (Pl. III, Fig. 4)

Large numbers of oogonia also undergo atresia *in situ*. The contained ovum with the granulosa layer shrinks away from the thecal covering. The ovum contracts in size and disappears leaving behind a group of granulosa cells in the follicular lumen. These turn pycnotic and degenerate, and a clear cavity is left behind surrounded by the thecal covering.

III. CYSTIC DEGENERATION

Cyst formation is rare in the ovary of the pigeon. Two kinds of cysts are met with :

(*i*) The oocyte shrinks away from the thecal covering and fibrosis takes place in it, resulting in the formation of a cyst (Pl. III, Fig. 5). Later the ovum breaks down and is destroyed by the growth of the stromal connective tissue.

(*ii*) Now and then, follicles are found with a central cavity devoid of any contents (Pl. III, Fig. 6). It looks as if the ovum liquefies and degenerates *in situ*, after which the granulosa cells are also lost resulting in the formation of a true cyst.

DISCUSSION

Brambell (1956) remarks that "follicular atresia is common and may be regarded as a process occurring regularly in the ovaries of vertebrates at all ages". Speaking of marsupial and placental mammals, Branca (1925) says : "En un mot, l'atrésie phénoméne anormal est la règle ; la pont, processus physiologique, l'exception". This applies equally in the case of the domestic pigeon also.

Bretschneider and De Wit (1947) classified the follicular derivatives found in vertebrates into (*i*) the post-ovulatory corpus leteum, (*ii*) the pre-ovulatory corpus luteum, (*iii*) the atretic corpus luteum, and (*iv*) the calyx. According to these authors, the difference between the pre-ovulatory and atretic corpora lutea (follicles) is that in the former the ovular debris is eliminated by the granulosa cells, and, in the latter by theca interna cells. Pearl and Boring (1918) also report that in the atretic follicles of the fowl, the granulosa cells degenerate and the cells of the theca interna hypertrophy and eliminate the yolk. The findings in the pigeon do not uphold this view. In the atretic follicles of the pigeon, the whole burden of elimination of the ovular debris lies on the granulosa cells. They hypertrophy, become phagocytic and ingest yolk globules. The theca interna cells do not play any part in the phagocytosis. Henneguy (1894), Dubuisson (1905) and Fell (1925) also have stressed the part played by the granulosa cells during atresia of avian follicles. Rowan (1930) and Davis (1942) attribute the disposal of the yolk to the phagocytes, but they say nothing about the origin of these cells. The active participation of the granulosa cells in the removal of the follicular debris during atresia has been reported from all classes of vertebrates (see Bragdon, 1952, for review). This observation points out that the fundamental mechanism of atresia is more or less similar among the vertebrates than was previously supposed.

The removal of the yolk extruded during bursting atresia of the follicles in the pigeon is partly carried out by phagocytes of granulosa origin and partly by phagocytes derived from the stroma. The origin of the phagocytes from the stroma of the ovary has not so far been reported in the case of birds. However, Garde (1930) reported the transformation of stroma cells into phagocytes in the ovary of *Ornithorhynchus*, during the bursting atresia of its follicles.

During atresia in the pigeon, the granulosa cells fill the lumen by hypertrophy only. This is in agreement with the findings of most workers, though Bretschneider and De Wit (1947) and Carlisle (1951) observed divisions of the granulosa cells during follicular atresia in *Rhodeus amauris* and *Ciona intestinalis* respectively.

A yellow pigment derived from the erythrocytes is reported to be present in the atretic follicles of the fowl by Pearl and Boring (1918) and Fell (1925). However, no pigment was found in the atretic follicles in the ovary of the pigeon, even though extensive haemorrhage was sometimes observed in several cases.

Atresia exhibits much variation not only in the different groups of vertebrates, but also in follicles of different sizes. In the pigeon, bursting atresia is characteristic of the larger heavily-yolked follicles. In birds (Dubuisson, 1905 ; Rowan, 1930 ; Wynne-Edwards, 1939 ; Davis, 1942 and 1944 ; Bullough, 1942 ; Moreau, Wilk and Rowan, 1947 ; and Marshall and Coombs, 1957), monotremes (Hill and Gatenby, 1926 ; and Garde, 1930) and reptiles (Loyez, 1906 ; and Bragdon, 1952) bursting atresia is the usual method of eliminating the ovular debris from heavily-yolked eggs. Recently Sathyanesan (1960) has recorded burst oocytes in the ovary of the fish, *Mystus seenghala*.

The bursting atresia resulting in the extrusion of yolk into the body cavity (Pl. II, Fig. 3) has been observed in the pigeon only in a few cases (Dominic, 1960*a*). Such atresia is of only limited occurrence in the avain ovary (Dunham and Riddle, 1942). Similar reports have been made by Garde (1930) in the case of *Ornithorhynchus*.

A freshly burst atretic follicle can be distinguished from the post-ovulatory follicle (Dominic, 1959) in sections by the presence of extruded yolk in the stroma in the first case. However, it is difficult to distinguish an old atretic follice from an old post-ovulatory follicle. This becomes more difficult to do with atretic follicles which have ruptured into body cavity.

The *in situ* atresia usually sets in immature follicles (Pl. III, Fig. 1). The type of atresia described in the oogonia (Pl. III, Fig. 4) does not seem to have been reported previously in birds. It is seen more frequently in 'resting' ovaries.

Cyst formation (Pl. III, Figs. 5 and 6) appears to be of only rare occurrence in the avian ovary (Dominic, 1960*b*) as it is seldom reported by previous workers. However, cystic degeneration of the follicles is common in the mammalian ovary (see Brambell, 1956, for review).

Blood follicles (Pl. II, Fig. 1) are also rare in the avian ovaries, (Dominic, 1960 *c*), though they commonly occur in mammals (see Brambell, 1956).

The high frequency of the atretic follicles is a very characteristic feature of the ovary of the pigeon. Why such a large number of atretic follicles occur in the ovary of this bird is not clear. Follicular atresia is seen not only in the ovary of the adults, but also in the ovaries of young onces and nestlings.

Speaking on the high frequency of follicular atresia in tropical African and tropical American birds, Moreau, Wilk and Rowan (1947) comment that it "represents an emergency supply of mature eggs, in preparation against failure of current nesting. When the season wanes and nesting is over, they become superfluous and are demolished by resorbing". This high frequency of follicular atresia, they believe, may be correlated with a low degree of nesting success from the abundance of reptilian and other predators. But this cannot be the only explanation of the high frequency of atresia in a domesticated bird like the pigeon which is less exposed to the ravages of predatory animals.

It stands to reason that, at the end of the breeding season, no useful purpose will be served by retaining the full grown and mature ova inside the ovary till the onset of the next breeding seasen, because the viability of the ova is most likely to be affected, and, may be, are therefore resorbed by atresia.

The total reproductive activties of a bird, including egg-laying, imply a drain upon its resources and the wear and tear from which the gonad suffers in the reproductive season has to be made good and preparations for the next season are

also to begin. The dissolution of the unused ova is, therefore, intended to release formative material for restitution and rehabilation of the gonad after the breeding season as well as to clear the ovary of an unnecessary charge.

The occurrence of atretic follicles in young birds can be explained by assuming that there is a kind of internal competition and struggle for existence between the several ova developing simultaneously in the ovary, each one of them struggling to attain maturity. In this struggle, some succeed and others fail, because sufficient nutrient matter is not available to all of them. And so atresia sets in them in order that the formative material locked up inside such follicles may be broken down and released for utilisation for future requirement.

It is somewhat difficult to explain atresia in the ovaries of nestlings. Here we will have to think in terms of genetics and assume that there is something wrong or something goes wrong with the genic inheritance of an oogonium in the course of maturation and vitellogenesis, so that instead of following the normal path of development, its future progress becomes arrested, when it tends to become destroyed and absorbed instead of turning into a necrotic focus within the ovary.

Bretschneider and De Wit (1947) were the first to attribute a function to the atretic follicular derivatives in vertebrates. They have shown that the atretic follicles (their 'pre-ovulation corpora lutea') in the bitterling, *Rhodeus amaurus*, secrete a hormone which induces the growth of the ovipositor. They named this hormone *oviductin* and think that the atretic follicles in reptiles, birds and oviparous mammals probably produce a hormone that serves to prepare the oviduct for shell formation.

Hooker and Forbes (1947) and Fraps, Hooker and Forbes (1948) found appreciable amounts of progestin in birds. Further Fraps (1955) reports a high threshold of progestin during ovulation. He found that experimentally administered progesterone induces ovulation, but not in hypophysectomised hens, which means that the production of a substance like progesterone is under the overall control of the pituitary. Breneman (1955) has evidence to show that both progestin as well as estrogen may be concerned in the seasonal hypertrophy of the oviduct in birds. Fraps (1955) suggests that an avian progestin, probably elaborated in the atretic follicle, might be concerned in the release of the ovum. He found progestin in the plasma of non-laying hens but not in the castrates. Marshall and Coombs (1957) have shown that in the ovary of the rook, *Corvus frugilegus* great many follicles undergo lipoidal atresia producing cholesterol which later disappears before any follicle matures sufficiently to discharge its ovum. Therefore they believe with Fraps (1955) that the pre-ovulatory luteoid (progesterone) is formed in the atretic follicles by their disintegration and a relatively high threshold of progestin is produced in the ovary in this way. They are of opinion that, it is not improbable that, in birds and other non-mammalisn vertebrates, the progestin necessary for inducing ovulation (cf. Fraps, 1955) is produced by the oft-repeated atresia of the follicles, whilst in viviparous mammals there has been a shift to a somewhat more stable progestin producing mechanism, *i.e.* the *corpus luteum*.

In the present state of our knowledge, it can only be said that probably the atretic follicles are not wholly inert bodies in the ovary of non-mammalian vertebrates, but play some kind of an endocrine role in them. Marshall and Coombs (1957) have rightly observed that a comparative cytological study of the atretic and the post-ovulatory follicles of selected viviparous non-mammalian vertebrates on the one hand and those of closely related oviparous species on the other, is destined to yield interesting results.

SUMMARY

1. The atretic follicles in the ovary of the pigeon are classifiable into (*i*) *bursting* atresia (*ii*) *in situ* atresia and (*iii*) *cysts*.

2. Atretic follicles are to be regarded as a normal feature of the ovary of the pigeon because of their high frequency of occurrence.

3. The ovular debris during atresia is removed by the granulosa cells acting as phagocytes. The theca interna cells do not play any part in the phagocytosis as claimed by Pearl and Boring (1918).

4. In the atretic follicles of the pigeon, neither mitosis nor amitosis of the granulosa cells has been observed, and, therefore, their multiplication is ruld out.

5. Pigment formation as reported by Pearl and Boring (1918) and by Fell (1925) in the atretic follicles of the fowl, has not been noticed in the case of the pigeon.

6. The origin of the phagocytes from the stroma of the ovary in the bursting atresia has not so far been reported in birds.

7. The type of atresia which sets in the follicles, containing little or no yolk, does not seem to have been reported previously in the case of birds.

8. Cystic degeneration of the follicles is of rare occurrence in the ovary of the pigeon.

9. The dissolution of the unused ova, after the breeding season is intended for restituting and rehabilitating the gonad as well as for lightening the ovary of an unnecessary charge.

10. The occurrence of atresia in the ovaries of nestlings can be explained as due to a genic imbalance of the oogonium.

11. The significance of the atretic collicles is not properly understood. May be that the progestin necessary for inducing ovulation (Fraps, 1955) is produced by the oft repeated atresia of the follicles. Thus the atretic follicles may be playing some kind of endocrinal role in *non-mammalian* vertebrates.

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PHYSIOLOGICAL STUDIES ON SALT TOLERANCE IN CROP PLANTS XIII. INFLUENCE OF IAA ON THE DELETERIOUS EFFECT OF SODIUM SULPHATE ON ROOT GROWTH IN WHEAT

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INTRODUCTION

Soil salinity has atained the status of major problem in agriculture particularly with increase in irrigation facilities in semi arid regions. Saline soils contain relatively high amount of total soluble salts of which sodium sulphate forms a major component. It may vary from 0·026% (in normal soil) to 0·269% (in saline soil) in Western U. P. (Desai and Sen, 1953). Root growth of plants appears to be the first target of the adverse saline conditions.

The depressing influence of sodium sulphate on root growth of tomatoes has been observed by Lyon (1941), Hayward and Long (1941 and 1943) and Eaton (1941) and the author (Sarin and Rao, 1956) also observed a similar effect of sodium sulphate on root growth of wheat and gram seedlings. On the other hand very low concentrations of IAA, when supplied continuously to the growing seedlings were observed to accelerate the root growth (Amlong, 1936; Fiedler, 1936; Thimann, 1937; Gross, 1940; and Ashby, 1951) while higher concentrations retarted the same. But a few workers (Marmer, 1937; Leonian and Lilly, 1937; and Kaiser and Album, 1939) failed to observe the stimulation even in similar low concentrations. However, it is not intended to give a detailed review on this aspect, as the present study relates to the after-effect of IAA.

The stimulatory behaviour of presowing soaking of the seeds with dilute solutions of IAA on the root growth has been noted by Thimann and Lane (1938) for Avena and Triticum, by Macht and Gumbein (1937) for *Lupinus albus*, by Hwang and Pearse (1940) for *Avena sativa* and *Vicia faba* and by Loo and Tang (1945) for maize and cabbage. But the use of phytohormones to recover the depression due to salts in seedling growth of plants apparently has not been reported so far. Therefore in the present study an attempt was made to investigate the possibility of counteracting the adverse effect of sodium sulphate on root growth of wheat seedlings by presowing soaking the seeds in a suitable concentration of IAA.

METHODS AND MATERIALS

Wheat C. 591 seeds were selected for uniformity of size and soaked (partly immersed) for 24 hous in varying concentrations (0·01, 0·05, 0·1, 0·5, 1·0, 3·0, 5·0 and 7·0 ppm) of β-indolylacetic acid (IAA) and the water soaked seeds were kept as control. After soaking, the seeds were washed with distilled water to remove the surface film of adhering solution and transferred to test tubes fitted with filter-paper rolls and filled nearly to one third with distilled water. Two seeds per tube were inserted between filter paper and the glass wall. The seeds were allowed to grow

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in humid dark chamber at about 30°C. Total root length was measured at 24 hours interval upto four days, but the final observations at the end of 96 hours were explained for this preliminary study.

When the 'stimulatory' concentration of IAA for root growth was established from the above studies, then a final experiment was conducted with seeds of wheat C. 591 soaked in the same (0·1 ppm solution of IAA) or distilled water ('control') for 24 hours and then seeds from both the sets were transferred to test tubes. Two series of tubes were maintained for each of the 'soaking' treatments, one containing distilled water and the other 0·6% sodium sulphate solution (a depressing concentration for root growth determined from earlier studies (Sarin and Rao, 1956) Daily observations were recorded upto 96 hours after sowing for total root length, main root length and number of roots and also the average root length was calculated for each seedling.

Ten replications were maintained for each treatment in the different experiments and the results were statistically analysed following analysis of variance for each duration. The distribution of degrees of freedom for the final experiment was as follows:

	D. F.
Due to 'Seed treatments'	1
Due to 'Salt supply'	1
Due to 'Interaction' ('Seed treatments' × 'Salt Supply')	1
Residual	36
Total	39

RESULTS

(*i*) Influence of IAA concentrations: The results for the various concentration of IAA were obtained in different experiments, as it was not possible to include all the concentrations in a single experiment. But, as each experiment had its own control the results for different concentrations are represented below as percentages on the respective controls.

TABLE I

Effect of different concentrations of IAA on root growth (in mms.) in 96 hours old wheat seedlings.

(% on respective controls)

Concentration in ppm							
0·01	0·05	0·1	0·5	1·0	3·0	5·0	7·0
—	—	—	—	—	—	—	—
116·6	108·1	120·5	113·5	96·1	103·7	89·0	88·6

The presowing soaking of seeds in IAA depressed the total root growth in concentrations even as low as 1 ppm; however, the inhibitive effect was statistically significant only for higher concentrations *i.e.* 5 ppm and 7 ppm.

IAA concentrations below 1 ppm enhanced the root growth but the acceleration was maximum for 0·1 ppm where the increase was 20·5% over the control (statistically significant). In the other low concentrations, the values fluctuated around 10% stimulation but the concentration giving the peak value (0·1 ppm) was selected for studying the influence of IAA on root growth of the seedlings growing in sodium sulphate solution (0·6%).

(*ii*) Influence of 'stimulatory' concentration (0·1 ppm) of IAA on the depressing effect of sodium sulphate solution (0·6%) on root growth :

TABLE II

Influence of presowing soaking of seeds in IAA on the depressing effect of sodium sulphate on root growth in wheat seedlings.

'Main factors'						'Interaction'				
'Seed Treatments'			*'Salt supply'*			*W*		*IAA*		
W	IAA	C.D.	C	N	C. D.	C	N	C	N	C.D.
Total root length (*mm*) :—										
48 hours after sowing :										
44·6	58·2	8·1	53·1	49·6	—	51·6	37·6	54·7	61·7	11·4
72 hours after sowing :										
142·4	172·3	17·9	163·6	151·1	—	153·8	131·8	174·2	170·4	—
96 hours after sowing :										
255·5	288·2	16·9	289·7	254·0	16·9	273·6	237·5	305·9	270·4	24·4
Main root length (*mm*) : —										
48 hours after sowing :										
23·2	26·6	2·8	25·6	24·2	—	26·3	20·1	35·0	28·3	4·2
72 hours after sowing :										
57·0	62·7	3·8	62·6	57·1	—	61·7	52·4	63·6	61·8	3·8
96 hours after sowing :										
87·2	93·5	4·4	98·2	82·6	4·4	96·3	78·2	100·1	87·0	—
Number of roots :—										
48 hours after sowing :										
2·9	3·0	—	3·0	2·9	—	2·9	2·9	3·1	2·9	—
72 hours after sowing :										
4·0	4·2	—	4·1	4·1	—	3·9	4·1	4·3	4·2	—
96 hours after sowing :										
4·2	4·5	—	4·2	4·5	—	4·2	4·3	4·3	4·7	—
Average root length :—										
48 hours after sowing :										
15·2	19·3	2·4	17·6	17·0	—	17·6	12·9	17·6	21·0	3·4
72 hours after sowing :										
33·2	39·1	3·4	38·2	34·1	—	36·9	29·4	39·4	38·9	—
96 hours after sowing :										
56·6	65·4	5·0	67·7	54·2	5·0	62·8	50·4	72·7	58·1	—

C : Distilled water supply.
N : Supply of 0·6% sodium sulphate solution.
W : Distilled water soaking.
IAA : 0·1 ppm IAA soaking.
C.D. : Critical difference at 5% probability.

Total root length: Total root length was adversely affected by sodium sulphate solution at all 'ages' of the seedlings but statistically significant reduction was observed only at the end of 96 hours. On the other hand IAA soaking enhanced total root length over water soaked controls, irrespective of 'salt supply' treatments.

The above results for the two main factors did not give a complete picture, as the interaction was also significant at 5% level, at least for 48 hours' and 96 hours' observations. Initially, *i.e.* at the end of 48 hours, total length was significantly depressed by sodium sulphate. IAA soaking resulted in similar total root length as that in water soaked controls. But, when IAA soaked seeds were supplied with sodium sulphate solution, the total root length was definitely better than distilled water soaked seeds in sulphate solution and was even better than both *i e.* its own and even distilled water soaked control. Similar trends were observed at 72 hours but IAA soaked seeds grown in sulphate solution could not result in better growth than their own controls and this tendency continued even upto 96 hours but the differences were, however, not significant. These facts clearly indicated that sodium sulphate (0·6%) was difinitely inhibitive for total root length but the depression could be nullified by IAA soaking for 24 hours.

Main root length: The length of the main root also followed trends similar to total root length. It was also similarly depressed by sodium sulphate, irrespective of 'seed treatments', but significant inhibition was observed only at 96 hours. However, IAA soaking enhanced the main root length at al 'ages' of the seedlings.

The interaction was significant at all 'ages' except at 96 hours. Like total root length, the main root length of distilled water soaked and sodium sulphate supplied seedlings was definitely lower than the 'controls' (distilled water soaked seeds growing in distilled water) but IAA soaked seeds under similar condition (*i.e.* with salt supply) resulted in main root length which was better not only from distilled water soaked and sodium sulphate supplied seedlings but also from water soaked and distilled water supplied seedlings and even IAA soaked and distilled water supplied seedlings. However, IAA soaked and sodium sulphate supplied seedlings were faring better than IAA soaked and distilled water supplied seedlings only at 48 hours' interval otherwise the latter were the best throughout.

Number of roots: The number of roots was not responding significantly to these treatments but sodium sulphate led to a slight reduction at 48 hours, which was not seen either at 72 hours or 96 hours, and in the last mentioned 'age' sodium sulphate actually tended to increase root production which was, however, not significant. IAA soaking improved the number of roots and the beneficial effect increased with the age of the seedlings. In general, IAA soaking appeared to have enhanced root production but the recovery etc. could not be accounted for because the depression due to the salt itself was not clear.

Average root length: Much like the total root length, the average root length was also decreased by sodium sulphate irrespective of 'seed treatments' but significant reduction was observed only after 72 hours. However, IAA soaking improved the average root length at all 'ages'. The interaction was significant only at 48 hours' interval meaning thereyby that both the factors were independant of each other after that period. But the inhibitive effect of sodium sulphate and its subsequent recovery by IAA soaking, bringing the average root length in IAA soaked and sodium sulphate supplied set better than even distilled water soaked 'controls',

was quite manifest (though statistical significances were there only for 48 hours' observation) IAA soaking alone, like total root length, was best except at 48 hours when too the difference was not significant. These results again proved conclusively that like total root length and main root length, average root length also behaved in a similiar manner to sodium sulphate and IAA.

The results on all these four observations of root growth convincingly proved the deleterious influence of sodium sulphate and the accelerating effect of IAA soaking and also, if the IAA soaked seeds were sown with a supply of sodium sulphate then the injurious effect of the salt was neutralised. The beneficial effect of IAA appeared to be both on root enlongation as well as root production while the injurious influence of sodium sulphate was mainly on root elongation.

DISCUSSION

According to several workers a continuous supply of low concentration of indolylacetic acid to growing plants or excised roots accelerate their root growth; the concentration apparently varied with the type of plant. At the same time, the absence of any stimulating effect on root growth in even low concentration of the phytohormones was also noticed by some workers, but all are agreed on the inhibitive effect of higher concentrations on root growth. However, relatively few workers attempted to study the effect of pretreatment of the seeds or roots of the seedlings or even excised roots with IAA solution on the later growth of the roots. Thimann and Lane (1938) as well as others (Macht and Gumbein, 1937; Hwang and Pearse, 1940 and Loo and Tang, 1945) observed a definite acceleration of root growth after the germinating seeds were supplied for a short duration with varying concentration of IAA; initially there was a depression followed by acceleration of root growth. However, the experiments of Loo and Tang (1945) were more in line with the present investigation wherein the seeds received a presowing soaking in IAA solution of different concentrations; the germinating seeds were then supplied with distilled water. In the present study, in concentration above 3 ppm, total root growth of 4 days old seedling amounted to only 89% of the control while four days old seedlings from seeds soaked in 0·1 ppm for 24 hours and allowed to grow in distilled water exhibited an increase of 20·5% over the root growth of the controls. It apparently supported the findings of other workers (Macht and Gumbein, Hwang and Pearse and Loo and Tang).

The conclusive evidence obtained in the present studies regarding the stimulation of root elongation as a result of presowing soaking of wheat seeds in 0·1 ppm of IAA encouraged to make use of this treatment to overcome the retarding effect on root growth of a fairly high concentration of sodium sulphate solution, which will be now considered in some detail.

The results on the influence of presowing soaking of seeds with IAA on the depressing effect of sodium sulphate solution (0·6%) on root growth of wheat were explained already, but strictly on the basis of statistical analysis and that too for the absolute performance at each interval. It is, however, worthwhile to consider the data on absolute growth and the rate of growth as percentages over the respective controls (distilled water soaked) for a better understanding of the effect of IAA on salt injury (Fig. 1).

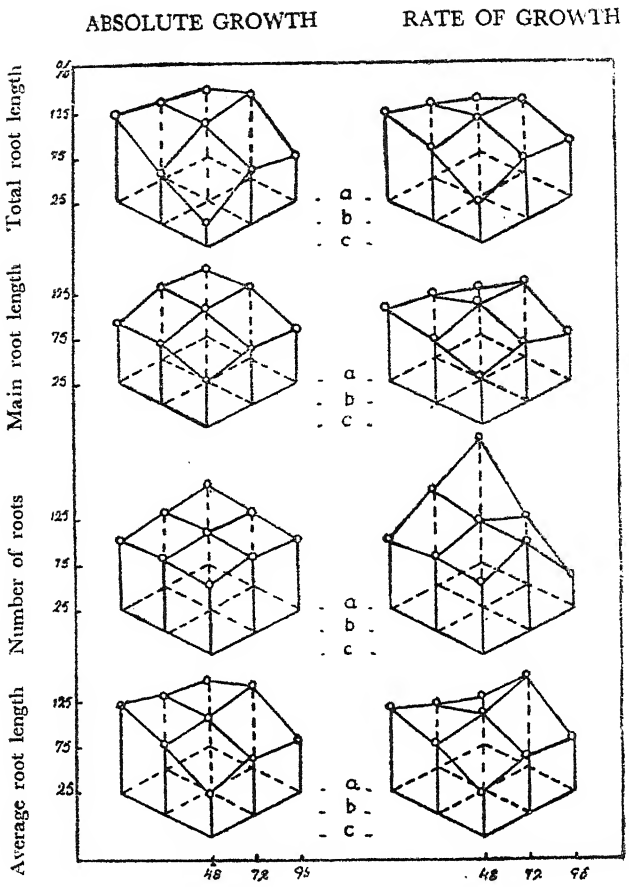

Fig. 1 : Influence of IAA on the depressing effect of sodium sulphate on root growth and its rate in wheat C. 591 seedlings.

(%over respective controls)

a : Distilled water soaked seeds grown in sodium sulphate solution (0·6%).

b : IAA soaked seeds grown in distilled water.

c : IAA soaked seeds grown in soudium sulphate solution (0·6%).

Total root length of distilled water soaked and sulphate supplied seedlings suffered a maximum reduction at 48 hours (27·2%) and it intended to improve at 72 hours and 96 hours. IAA soaked seeds growing in sulphate solution were exhibiting better total root length than distilled water soaked but unlike water soaked set there was no recovery with increase in age, on the other hand it decreased with time but even at 96 hours it was fairly close to distilled water soaked 'controls' while that of the water soaked set (growing in sulphate solution) was always lower than 'controls'. The rate of root growth also exhibited similar trends *i.e.* a recovery in water soaked seeds with increase in age upto 72 hours after which it once again

went down while in IAA soaked seeds there was a continuous reduction in rate with increase in age, so much so that the rate at 96 hours was depressed more in IAA soaked seeds (16·5%) as compared to distilled water soaked seeds (11·5%.

The main root length of seedlings, raised from distilled water soked seeds, growing in sulphate solution, was also retarted as compared to 'controls' and the depression was more pronounced at 48 hours and showed a recovery which, unlike total root length, however, did not continue upto 96 hours and was evident only upto 72 hours after which once again the curve tended to go down. IAA soaked seeds growing under similar conditions *i.e.* with a supply of sodium sulphate, had main root length better than distilled water soaked controls at 48 hours but from then it gradually went down, however, unlike water soaked seeds in sodium sulphate solution it remained always close to the 'controls'.

The rate of main root length elongation in seedlings from water soaked seed growing in sodium sulphate solution was also retarted as compared to 'controls' (distilled water soaked seeds growing in distilled water) but the retardation was not constant throughout and showed a recovery between 48 and 72 hours after sowing but between 72 hours and 96 hours once again it went down and reached even lower than the 48 hours' value. IAA soaking (with a supply of sodium sulphate) resulted, though in a better rate than 'controls' at 48 hours, but later on it was always lower than control and the lowering was also more marked at 96 hours than at 72 hours. At 96 hours, depression in rate of growth was similar in both the soaking treatments (distilled water and IAA soaking).

Root production was not adversely effected by the salt supply to distilled water soaked seeds or IAA soaked seeds. The rate of root production, though similar to 'controls' at 48 hours after sowing in distilled water soaked and sodium sulphate supplied seedlings, increased between 48 and 72 hours (20% over control at 72 hours) but later on (*i.e.* 96 hours after sowing) went down to even lower values than that of the 'controls.' Like water soaked seeds, the IAA soaked seeds growing in salt solution also had similar rate of root production as the 'controls' at 48 hours after sowing but unlike the former they continued to be better than controls throughout the experimental period so that at 96 hours when the rate of water soaked set was depressed by sodium sulphate it was enhanced by IAA soaking.

The average root length of water soaked and sulphate supplied seedlings was also lower than 'controls' and the maximum reduction was observed at 48 hours after sowing followed by a recovery which persisted upto 96 hours, but always it was lower than 'controls'. IAA soaking brought about an initial increase over the 'controls' but the stimulatory effect decreased with the age of the seedlings, however, even at 96 hours the reduction due to the salt supply in IAA soaked seedlings was relatively lesser (7·5%) than distilled water soaked seedlings (19·8%).

From the above consideration it is fairly evident that a supply of sodium sulphate solution depressed the total root growth of wheat seedlings raised from distilled water soaked seeds and the deleterious effect appeared to be largely on the phenomenon of root elongation rather than rooting as the number of roots remained unaffected, thus confirming the earlier findings of the author (1959). IAA soaking under similar conditions (*i.e.* with salt supply) resulted in root growth definitely better than distilled water soaked seeds. Further, the stimulatory effect of IAA on root elongation as well as on the root production.

Although the present study offers a new line for methods to improve growth of plants in salt-lands, it should be noted that the present results pertain to root growth of only four days old seedlings and the beneficial effect of IAA may

disappear gradually. Perhaps the relatively more concentrated supply of this or other similar synthetic phytohormones in the beginning or spraying of the growing plants with suitable concentration or their addition to the soil may be found useful. The author has not come across any investigation on these lines except his own reported elsewhere.

SUMMARY

Earlier studies indicated that root growth of wheat seedlings was adversely affected by a supply of sodium sulphate. The present investigation was undertaken with a view to utilize the stimulating influence of low concentration of IAA in counteracting the retarding effect of sodium sulphate.

In the beginning the seeds were subjected to presowing soaking for 24 hours in different concentration (0·01 0·05, 0·1, 0·5, 1·0, 3.0, 5·0 and 7·0 ppm) of IAA to ascertain the 'stimulatory' concentration for root growth, 5 ppm and above of IAA retarded root growth by about 20%.

Two lots of seeds were soaked for 24 hours, one in the 'stimulating' concentration (0·1 ppm) of IAA and the other in distilled water and sown separately in distilled water and 0·6% sodium sulphate solution. Total root length, main root length, number of roots and average root length were recorded at 24 hours interval and data was analysed statistically.

Total root length was depressed by sodium sulphate solution but IAA soaking resulted in root growth equal to control. IAA soaked seeds with salt supply exhibited total root length better not merely than water soaked and salt supplied seedlings but also from water soaked 'controls' at each interval (48, 72 and 96 hours after sowing).

The length of the main root as well as average root length also showed similar trends and the nullifying effect of IAA soaking was further borne out. The root production was not retarded by the salt supply but was enhanced by IAA soaking. Thus the IAA effect appeared to be on both rooting as well as root elongation, collectively resulting in overcoming of the deleterious influence of sodium sulphate on root growth.

The possibility of improving growth of plants in saline areas by using suitable concentrations of IAA has been indicated.

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THE URINOGENITAL ORGANS OF *MALLAGO ATTU* (BL. & SCHN.)

By

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INTRODUCTION

As the Indian freshwater shark-*Wallago attu* is a type of bony fish suitable for class study, it has been considered desirable to make available a detailed account of its morphology. In two earlier papers (1959, 1961) the endoskeleton of the fish was described and the present paper is devoted to the urinogenital organs.

For the excretory organs in fishes references may be made to Marshall and Smith (1930) on the glomerular development of the kidney, Smith (1931) on the regulation of composition of blood and the evolution of vertebrate kidney, Storer (1932) on the development of pronephros in perch, and Moghe (1945) on the development of mesonephros in *Thynnichthys*.

In connection with the reproductive organs of fishes, attention may be drawn to Mckenzie (1884) on the urinogenital system of *Ameiurus catus*, Bennington (1936) on the origin of germ cells and spermatogenesis in *Betta splendens*, Bullough (1939) on the reproductive cycle in minnow, Purser (1938) on reproduction in *Lebistes reticulatus*, and Jones (1940) on histological changes in the testis in sexual cycle of male *Salmo salar*.

The pseudocopulatory papilla was described by Day (1889), who noted it in *Mystus keletius* and *Mystus armatus*. Mukerjee, Mazumdar and Das Gupta (1940) described it in *Mystus gulio* and the same authors (1941) in *Clarias batrachus* and *Heteropneustes fossilis*. Hora and Law (1941) made a reference to it in *Mystus malabaricus* and in species of *Batasio* and *Gagata*.

MATERIAL AND TECHNIQUE

Specimens of *Wallago attu* were procured from the local fish market for investigation. Besides gross dissections, serial sectioning of the kidneys, ureter, urinary bladder, testis, vas deferens, ovary, oviduct and pseudocopulatory papilla was also done. The slides were stained with Iron haematoxylin, Delafield's haematoxylin and cosin or Mallory's triple stain.

GENERAL

In vertebrates the excretory and reproductive organs are closely associated and are dealt with together. In the fish the association is restricted to the pseudocopulatory papilla, through which both the excretory and generative products escape out. In males, where there is a common orifice for the two products, the association may be said to be more intimate than in the females in which independent apertures exist for the products.

The sexes are separate and the only sexual dimorphism in the species is in the form of the pectoral fin and the pseudocopulatory papilla. The spine of the pectoral fin is more strongly developed and prominently indented along the inner side in males than in females. The pseudocopulatory papilla is slender and pointed in the males, while it is broadly built and bluntly rounded at its apex in females.

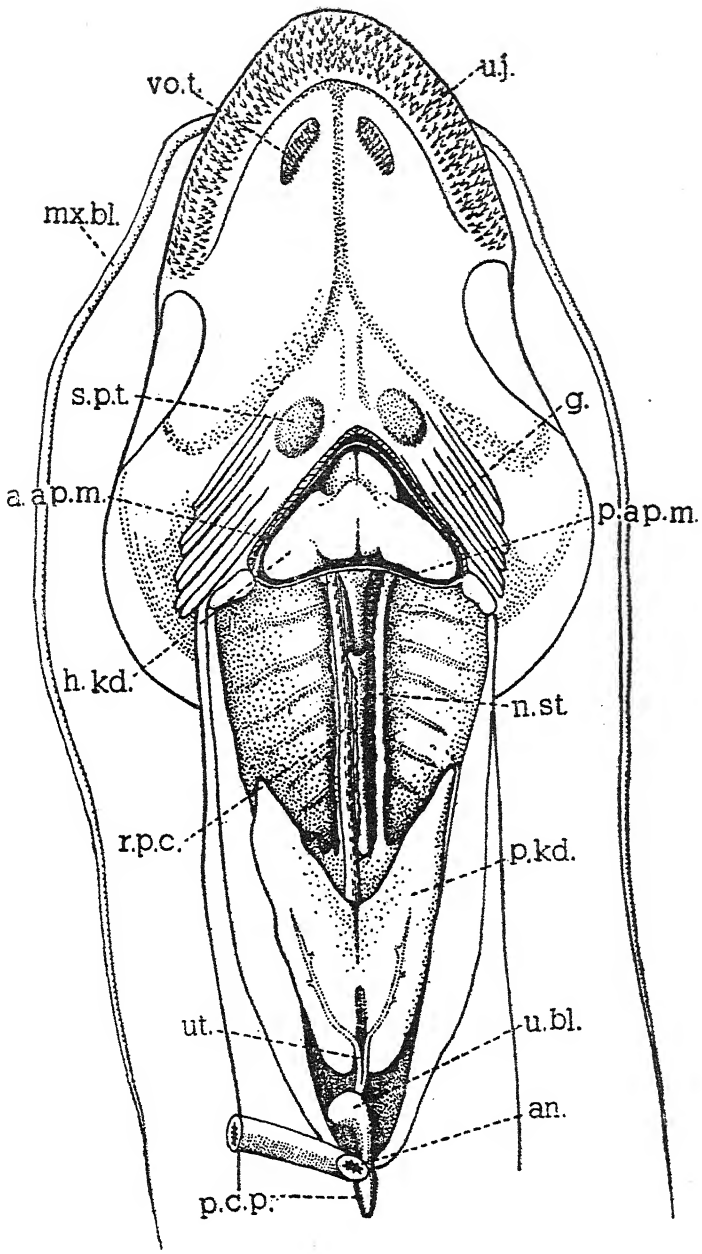

PLATE I

The Excretory Organs ($\times\frac{4}{5}$)

a.ap.m., anterior aponeurotic membrane ; *an.*, anus ; *g.*, gill ; *h.kd.*, head kidney ; *mx.bl.*, maxillary barbel ; *n.st.*, nephrogenous strand ; *p.c.p.*, pseudocopulatory papilla ; *p.ap.m.*, posterior aponeurotic membrane ; *p.kd.*, posterior kidney ; *r.p.c.*, right posterior cardinal vein ; *s.p.t.*, superior pharyneal teeth ; *u.bl.*, urinary bladder ; *u.j.*, upper jaw ; *u.t.*, ureter ; *vo.t.*, vomerine teeth.

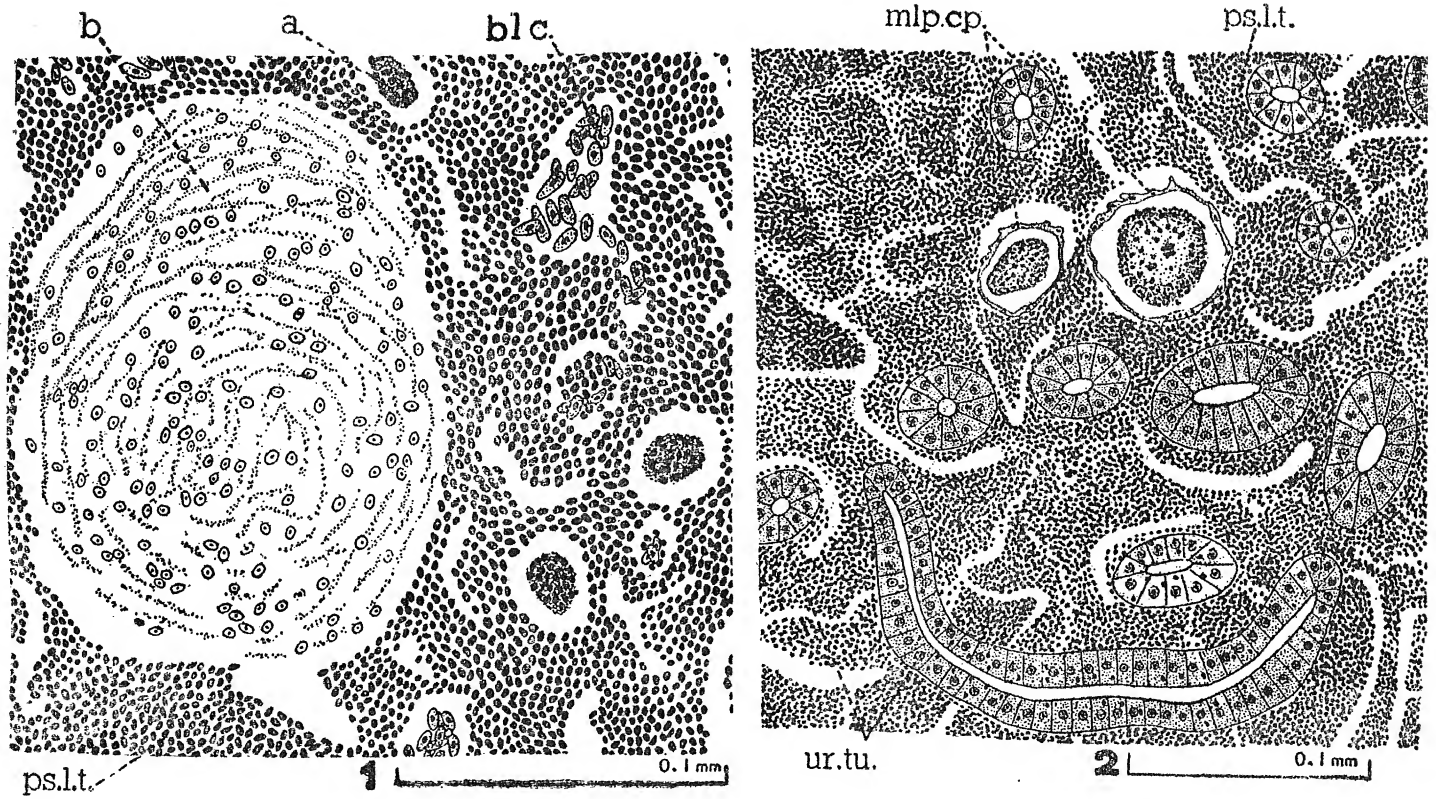

PLATE II

Fig. 1. Transverse section of head kidney ; Fig. 2. Transverse section of posterior kidney.

a., cavities with deeply stained cells ; *b.*, compact mass of lightly stained cells ; *bl.c.*, blood corpuscles ; *ml.cp.*, malpighian capsules ; *ps.l.t.*, pseudolymphoid tissue ; *ur.tu.*, urinary tubule.,

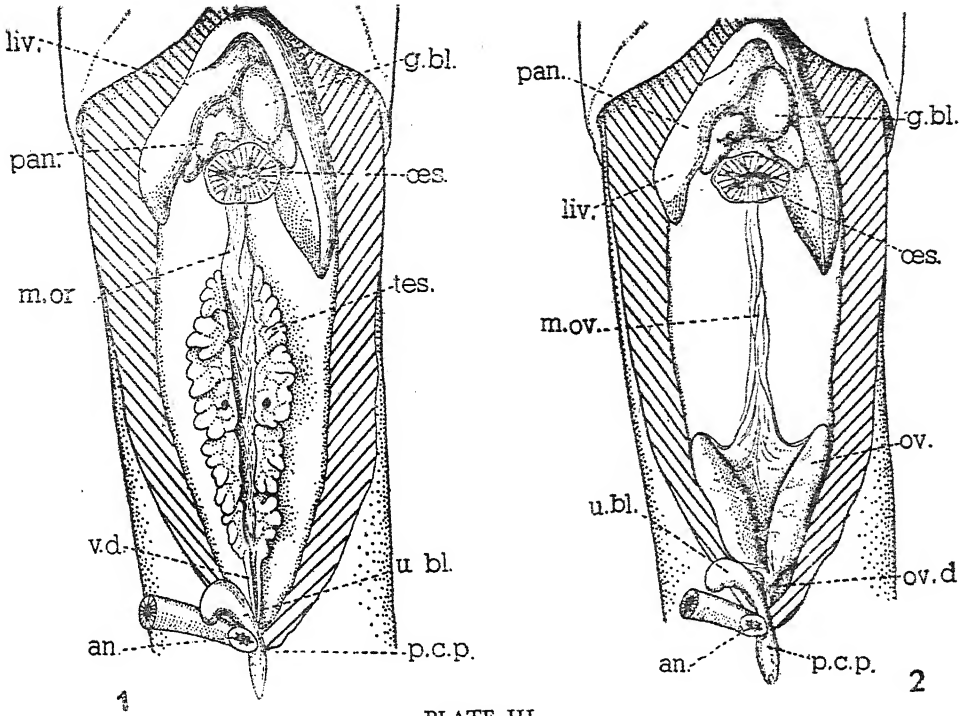

PLATE III

Fig. 1. Male Reproductive Organs ($\times\frac{3}{4}$) ; Fig. 2. Female Reproductive Organs ($\times\frac{3}{4}$).
an., anus ; *g.bl.*, gall bladder ; *liv.*, liver ; *m.orc.*, mesorchium *m.ov.*, mesovarium ; *oes.*, oesophagus ; *ov.*, ovary ; *ov.d.*, oviduct ; *pan.*, pancreas ; *p.c.p.*, pseudocopulatory papilla ; *tes.*, testis ; *u.bl.*, urinary-bladder ; *v.d.*, vas deferens.

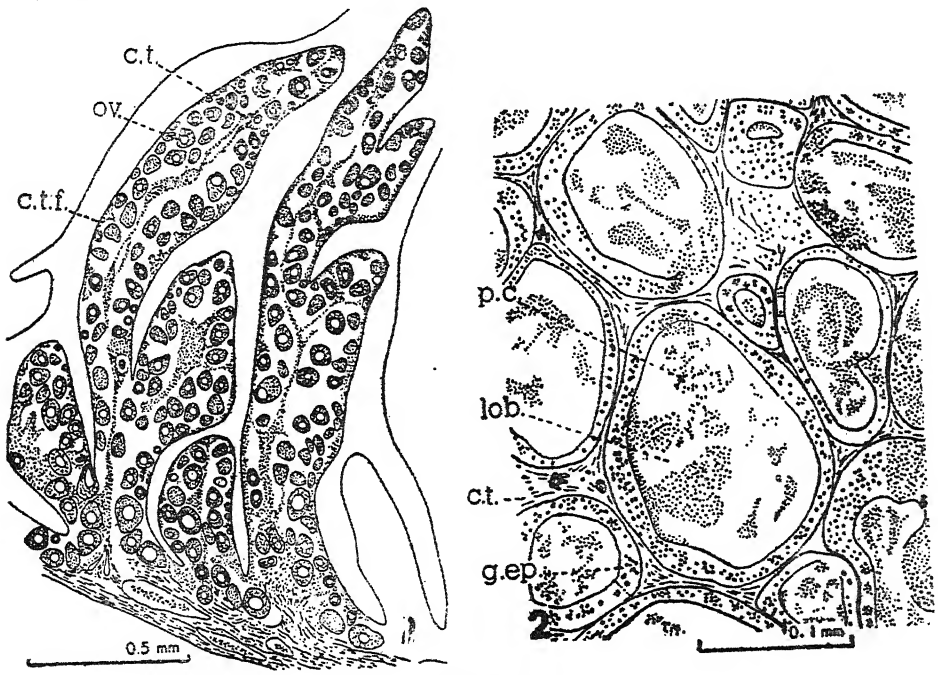

PLATE IV

Fig. 1. Transverse section of mature testis ; Fig. 2. Transverse section of ovary.
c.t., connective tissue ; *c.t.f.*, conective tissue fold ; *g.ep.*, germinal epithelium ; *lob.*, lobule ; ov., ovum ; *p.c.*, proliferated cells.

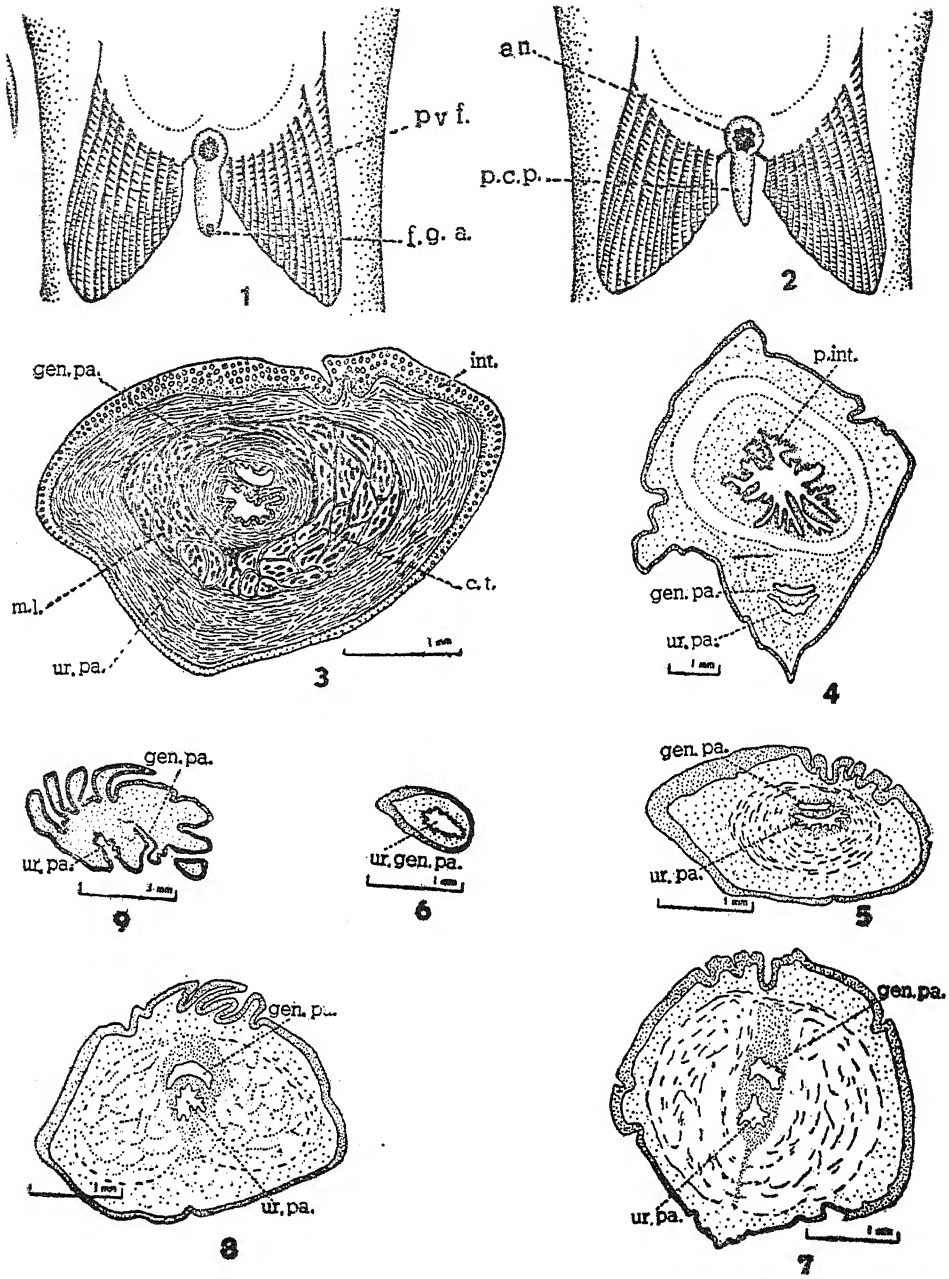

PLATE V

Fig. 1. Female Pseudocopulatory Papilla ; Fig. 2. Male Pseudocopulatory Papilla ; Fig. 3. Transverse section of Pseudocopulatory Papilla ; Figs. 4-6. Male Pseudocopulatory Papilla in serial transverse section ; Figs. 7-9. Female Pseudocopulatory Papilla in serial cross section.

an., anus ; *c.t.*, connective tissue *f.g.a.*, female genital a perture ; *gen. pa.*, genital passage ; *int.*, integument ; *m.l.*, muscular layer ; *p.c.p.*, pseudocopulatory papilla ; *p. int.*, posterior intestine ; *ur.pa.*, urinary passage ; *ur.gen.pa.*, urinogenital passage.

The Kidneys

There is a pair of kidneys (Fig. I), which are intimately fused together. Each is distinguished into a nonexcretory head kidney and an excretory posterior kindey. The *head kidney* (Pl. 1, h. kd.) lies in front of the air bladder in between the anterior and posterior aponeurotic membranes. The *anterior aponeurotic membrane* (Pl. I, a. ap. m.) is arched. The apex of the arch lies over the basioccipital, while the two ends extend out and fuse with the ends of the anterior divisions of the parapophyses of fourth vertebra. The *posterior aponeurotic membrane* (Pl. I, p. ap. m.) runs transversely at the level of the hind end of the second vertebra. Its two ends fuse with the terminations of the anterior divisions of parapopyses of the fourth vertebra, almost at the insertion of the ends of the anterior aponeurotic membrane. In the triangular space between the two membranes are contained the head kidneys, each convex on the dorsal side and flat on the ventral and indistinguishably fused in the middle line with one of the other side.

In a transverse section the *head kidney* (Pl. II, Fig. 1) is seen to consist of a closely packed mass of small oval cells rich in blood capillaries, which form the *pseudolymphoid tissue* (Pl. II, Fig. 1, ps. l. t.). There are no urinary tubules and no malpighian capsules. Scattered in the pseudolymphoid tissue are a large number of rounded cavities (Pl. II, Fig. 1, a) filled with deeply stained cells. In the peripheral region of the tissue are a few well developed rounded bodies, each formed of a compact mass of very lightly stained oval cells (Pl. II, Fig. 1, b).

The *posterior kidneys* (Pl. I, p. kd) lie behind the air bladder and extend back nearly to the hind end of the body cavity. Each kidney is convex on the dorsal and flat on the ventral side. In conformity with the lateral boundary of the body cavity, its outer border is curved, while the inner border with which it fuses with the one of the other side is straight. The fused mass of the two posterior kidneys is more or less conical, with the anterior end broad and the posterior narrow and rounded. The anterior face is deeply concave and its two lateral ends extend much forward up to half the length of the body cavity. Along the anterior face it is deeply grooved to enclose the hind end of the air bladder. The groove is so deep as to divide this end into a dorsal and a ventral part, which extend over the upper and lower surfaces of the bladder. The dorsal part is specially well developed and covers the posterior one-third of the air bladder. From this part arise strands of nephrogenous tissue, which extending forward on either side of the vertebral column connect the posterior and head kidneys of the same side. The kidneys are covered on their ventral face by a layer of peritoneum.

The *posterior kidney* (Pl. II, Fig. 2) in a transverse section is observed to contain a large number of *urinary tubules* (Pl. II, Fig. 2, ur. tu) cut in various planes, which are held in the pseudolymphoid tissue (Pl. II, Fig. 2, ps. l. t). Interspersed with the tubules lie the *malpighian capsules* (Pl. II, Fig. 2, mlp. cp.) and blood capillaries. The tubules are lined with a single layer of columnar glandular cells, which rest on structureless basement membrane. The malpighian capsuless* are round spaces lined with flat scale-like cells. Each space contains a deeply staind mass, which represents the *glomerulus*.

The Ureters

Through a notch between the two posterior kidneys and a little in front of their hind ends emerge two fine tubular *ureters* (Pl. I, u. t.). Each arises in its

*The kidneys in bony fishes may be with or without the malpighian capsules. According to Marshall and Smith (1930) the development of the malpighian capsules is related to water excretion and therefore, their development is better in freshwater forms. Smith (1931) stated that fishes in freshwater are hypotonoic in excretion and in marine habitat hypertonic in excretion.

kidney a little in front of the middle and extends back through it receiving collecting tubules all along its course. Beyond the kidneys the ureters run together to the ventral side and unite before they open in the urinary bladder. The ureters, which are pronephric ducts, are thick-walled and have a narrow lumen. Each is formed from inside out of a layer of epithelium, a sheath of connective tissue and a covering of peritoneum. The epithelium comprises of a single layer of columnar cells, which rest on a structureless basement membrane. The connective tissue-sheath has its fibres disposed circularly and includes blood capillaries. The peritoneum is formed of a single layer of flat cells.

The Urinary Bladder

The distal parts of ureters form the *urinary bladder* (Pl. I, u. bl.). It is a thick-walled dilated sac, which resembles an inverted retort flask ; its broad head lies to the right of the middle line and its neck extends medially to the hind end of the body cavity. At the junction of the head and neck opens the common ureter. The bladder is folded internally, the folds being more prominent in the region of the head than in the neck. The bladder comprises of the same three layers as the ureter. The lining epithelial layer, however, contains goblet cells in addition to the usual epithelial cells. At the hind end of the body cavity the neck of the bladder enters the genital duct and the urinogential duct passes into the pseudocopulatory papilla (Pl. I, p. c. p.).

THE MALE REPRODUCTION ORGANS

The Testes

There is a pair of closely placed and parallel running *testes* (Pl. III, Fig. 1, tes.), which lie on the ventral side of the air bladder and posterior kidneys held to them by mesorchium. The testes vary much in size during the year. The are full of spermatic fluid before spawning and are very prominent, but after spawning they get reduced and appear as a pair of inconspicuous strands. When fully mature the testes are elongated and strap-shaped and they extend from about the middle of the body cavity to a little behind the kidneys. Each testis is broad in the middle and gradually narrows towards its two ends. Its outer border is curved and inner straight. The testis is divided imperfectly into lobes by transverse fissures, which run inwards from its outer margin. The lobes are large in the middle of the testis and gradually get smaller towards the two ends. Each lobe is produced towards the outer border of the testis into finger-shaped processes, which run in a dorsal and a ventral series.

The *testis* is acinous and in a cross section (Pl. IV, Fig. 1) is found to consist of a large number of lobules (Pl. IV, Fig. 1, lob.) with very little of interlobular space. Each lobule is lined with germinal epithelium. The germinal epithelium is stratified at places due to the proliferation of cells (Pl. IV, Fig. 1, p.c.) and the lobules contain cells in various stages of spermatogenesis. The interlobular space is filled with connective tissue and contains blood capillaries.

The Vasa Deferentia

Towards the posterior end each testis opens into a slender tubular *vas deferens* (Pl. III, Fig. 1, v. d.), which is a continuation of the peritoneum covering the testis. The vas deferens is double layered, formed of an inner layer of epithelium and an outer layer of connective tissue. The epithelium is formed of more or less flat cells and the fibres of connective tissue layer are circularly disposed. The two vasa deferentia join below the urinary bladder to form the common genital duct, which extends back underneath the bladder and fuses with its neck at the hind end of the body cavity.

The ovaries

There is a pair of *ovaries* (Pl. III, Fig. 2, ov.) placed obliquely below the air bladder and posterior kidneys with their anterior ends wide apart and the posterior ends approximating. They are covered by the peritoneum and are attached to the air bladder and kidneys by the *mesovarium* (Pl. III, Fig. 2, m, ov.). The size of the ovaries varies during the year. Immediately after oviposition they become reduced and lie at the hind end of the body cavity in the form of a pair of diverticula. When fully mature, they are elongated sacs distended enormously by the ripe eggs and occupy the posterior two-third of the body cavity.

In a transverse section (Pl. IV, Fig. 2) the *ovary* appears as a sac of connective tissue, which contains elastic fibres and is covered externally by the peritoneum. From the inner wall of the ovarian sac arise folds, which are disposed in rings one behind the other hanging freely into the lumen. Each fold (Pl. IV, Fig. 2, c. t. f.) contains ova in different stages of maturity, the younger ones being towards the periphery. A mature ovum has a prominent nucleus in which are a number of nucleoli. Along the periphery of the folds among the ova are a few very tiny oognia, each completely filled by its nucleus containing a single nucleolus.

The Oviduct

A little behind the kidneys, the two ovaries unite and the peritoneum covering them extends back as the *common oviduct* (Pl. III, Fig. 2, ov. d.). The oviduct is a dilated tube, which at the posterior end of the body cavity fuses with the neck of the bladder. In histological details it resembles the vas deferens.

THE PSEUDOCOPULATORY PAPILLA

A small conical *pseudocopulatory papilla* (Pl. V, Figs. 1 & 2) lies between the pelvic fins behind the anus. It is thick and muscular and through it traverses the urinogenital duct separated by a transverse septum into a dorsal urinary and a ventral genital passage. The septum terminates a little before the apex of the pseudocopulatory papilla in the males and the urinary and genital passages open out by a common urinogenital aperture. In females the septum extends to the very end of the papilla and the two passages lead out independently, the urinary apertue being terminal and genital subterminal.

In cross section (Pl. V, Figs. 3-9) the papilla is found to be covered by the integument (Pl. V, Fig. 3, int.). It is composed of connective tissue (Pl. V, Fig. 3, c. t.), partly derived from the dermis of integument and partly from the connective tissue sheath of urinogenital duct. In between the two parts of the connective tissue is a thick layer of longitudinally disposed muscle fibres (Pl. V, Fig. 3, m. l.). The urinary passage (Pl. V, Fig.3, ur. pa.) is involuted and lined with columnar cells, while the genital passage (Pl. V, Fig. 3, gen. pa.) is smooth and lined with more or less flat cells. The septum between the two passages is formed of transversely disposed connective tissue fibres, which at the ends terminate and become continuous with the connective tissue of the papilla.

SUMMARY

1. The paired kidneys are indistinguishably fused. Each consists of a head kidney in front of the air bladder and a posterior kidney behind it, which are conected by strands of nephrogenous tissue. The head kidney is non-excretory and is without urinary tubules, while the posterior kidney is excretory and contains the urinary tubules and malpighian capsules.

2. The ureters are pronephric ducts, which get secondarily connected with the kidneys. They fuse before opening into the urinary bladder.

3. The paired gonads lie below the posterior kidneys. The testis is strap-shaped and divided into lobes produced towards the outer side into processes. The lobules are lined with germinal epithelium, the cells of which proliferate and undergo spermatogensis. The ovary is a sac of connective tissue produced into folds towards the cavity of the sac, which run in parallel rings. The folds contain mature ova towards the centre and young ones on the periphery.

4. The genital ducts are the continuation of the peritoneum covering the gonads. A single oviduct is present in the female, while there is a pair of vasa deferentia in the male.

5. A pseudocopulatory papilla is present behind the anus. The papilla is traversed by the urinogenital duct separated into a dorsal urinary and a ventral gential passage. The urinary passage is involuted and is lined with columnar cells, while the genital passage is smooth and is lined with flat cells. The urinary and genital passages open independently in female and by a common aperture in male.

6. The sexes are separate and sexual dimorphism exists to some extent. The spine of the pectoral fin is more strongly developed and more deeply indented in the male than in the female. The pseudocopulatory papilla is slender and pointed in the male, while it is broadly built and truncated at the apex in the female.

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PRE-EMERGENCE INJURIES CAUSED BY THE MICROFLORA OF STORED RICE, PEA AND GRAM SEEDS AND BENIFICIAL EFFECTS OF FUNGICIDAL SEED-DRESSING*

By

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I. INTRODUCTION

Many fungi are carried exclusively through seed and cause serious plant diseases. In the godowns, during storage, saprophytic fungi often cause seed rotting if the moisture and temperature conditions are favourable for their growth. Moreover when such defectively stored seed is sown, the soil inhabiting fungi cause further seed rotting and pre-emergence injuries. Literature from foreign countries shows that a protective covering of the seed by fungicidal substances prevents seed rotting and is generally helpful in establishing a healthy and vigorous growth of the seedlings. In India such studies had been comparatively few. Gopal Swarup (1950) studied the fungus flora of the stored wheat, oat and maize seed and the damage caused by some of the seed-borne fungi. When seeds were treated with various fungicides, the pre-emergence injuries were appreciably checked and the seedlings showed a good stand. Murthi (1951) carried out similar studies with sorghum and barley seed, and the authors extended these studies on rice, pea and gram seeds which form the subject matter of this paper.

MATERIAL AND METHODS

Ten strains of rice, seven strains of pea and five strains of gram were selected for the investigation. A number of N. P. (New-Pusa) varieties were included in the tests, rice varieties 97, 125, 130, 137 were obtained from the Karnal Sub-station of the Indian Agricultural Research Institute, pea varieties 18, 29, 33, 36 and gram varieties 17, 28 and 28 as well as Hoshiarpur 67/a, Delviche-commando and Zelka rice were obtained from the Botany Division of the Indian Agricultural Research Institute, New Delhi. Type 3 rice was obtained from the Rice Research Station, Nagina and Strains *Banjari, Basumati, Batro Ratnachudi* and CH_{47} from the Department of Agriculture, Bhopal. One local strain of pea and two local strains of grams *viz. Kabuli white* and *Local brown* were obtained from Khari-baoli market in Delhi.

The fungi associated with rice, pea and gram seeds of each variety or strain were studied both by examining the centrifuged seed washings and by making isolations of fungi from surface sterilized and unsterilized seed on 2 percent malt agar, 2 percent potato dextrose agar adjusted to pH 4·5 and 7·5 and Smith Humfeld agar.** For each sample four Petri plates were used and each test was repeated three times.

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**Sodium nitrate ...	1 gm.	Agar Agar ...	20 gm.
Potassium hydrogen phosphate	1 gm.	Distilled water to make	1,000 c.c.
Dextrose ...	10 gm.		

The role of the predominant fungi in relation to the germination and vigour of rice, pea and gram seedlings was studied. Predominant fungi were isolated and single spore cultures multiplied in 500 ml. Erlenmeyer flasks on 5 percent maize mael medium of the following composition :

Maize meal	10 gm.
Finely sieved soil	190 gm.
Distilled water	70 ml.

The inoculum thus obtained was mixed with the top 3 inches of sterilized soil filled in 10 inch diameter pots and was used at the rate of 250 gm. per pot and seeds sterilized with 0·1 percent mercuric chloride solution and subsequently washed with sterilized distilled water and sown in 2 sets of 9 pots filled with steam sterilized soil. In one set unsterilized seed was sown and in the other set sterilized seed was sown. Besides emergence, the weight and height of seedlings were also recorded to signify the vigour of the seedlings under each treatment.

The benificial effects of the fungicidal seed dressings were studied by 50 gms of pea N. P. 29 and gram N. P. 58 and 30 gm. of rice N. P. 130, with 12 fungicides for 15 minutes in separate Erlenmeyer flasks. A few treated and untreated seeds were plated on 2 percent potato dextrose agar (pH 4·5) and the rest of them were sown in 20 × 16 × 4 inch window pane boxes filled with finely sieved field soil. The seeds were sown 3 inches apart in 5 rows. Final emergence counts were taken on the twelvth day and after sowing after the lapse of a few days more 50 seedlings of rice and gram and 30 seedlings of pea were selected at random, washed free of soil, dried between sheets of blotting paper and their average height and weight determined. The experiment was repeated three times.

III. EXPERIMENTAL

(*a*) *Fungus flora associated with the stored rice, pea and gram seed.*

Sawada (1919), Suzuki (1930) and Tullis (1936) have reported from Formosa, Japan and Southern United States respectively that *Fusarium heterosporium*, *Piricularia oryzae*, *Helminthosporium oryzae*, *Trichoconis candata*, *Curvularia lunata*, and some unidentified species of *Fusarium* and *Phoma* were found associated with stored rice seed. In India, Padwick and Ganguly (1945), Padmanabhan (1949) and Ghosh (1951) report that *Cochliobolus miyabeanus*, *Curvularia lunata*, *Trichochonis candata*, *Nigrospora oryzae*, and some unidentified species of *Aspergillus* and *Penicillium* have been associated with rice seed. However, in these studies *Fusarium sp.* was observed in seed washings of all the varieties of rice, and was also the predominant fungus in isolations. *Alternaria sp.* was isolated from N. P. 130, N. P. 137, T3 CH 47 and *Helminthosporium sp.* was mostly associated with the seed of CH 47.

Padwick (1938) reported complex fungal rotting of pea seeds and isolated *Acromoniella atra*, *Chaetomium sp.* *Fusarium cinerea* and common moulds in England, where groves bud Skolko (1946) found *Acromoniella atra*, *A. verrucosa*, *Cladomyces-palmarum* and *Trichocladium asperum* as the predominant seed—borne fungi of pea.

In the present studies *Fusarium sp.* was isolated from all the samples used in our experiments. From Italy Delcanizo (1927) reports the associaton of *Phyllosticta rabeii* (*Mycosphaerella pinodes*), *Rhizoctonia solani* and *Ascochyta pisi* on stored gram seed, but in our studies *Alternaria sp.* *Mycogone sp.*, *Aspergillus sp.* and *Fusarium sp.* were the common fungi isolated.

(b) Role of predominant fungi in relation to the germination and vigour of rice, pea and gram seed.

Single spore cultures of the predominant *Fusarium sp.* isolated from rice and pea were used as test organisms. *Alternaria sp.* was used for gram. Sets of 9 pots 10″ in diameter were sown with unsterilized and stetrilized seed of rice, pea and gram respectively. The experiments were repeated twice.

Table 1. Gives a summary of the consolidated data on these experiments.

TABLE 1

Role of *Fusarium spp.* on the emergence and vigour of rice and pea seedlings and of *Alternaria sp.* on gram seedlings

Treatments		Percent emergence	Weight of seedlings in gm.	Height of seedlings in cm.
Untreated seed in Sterilised soil	Rice	72	3·8	13·9
	Pea	77	22·8	5·7
	Gram	93	15·6	8·5
Untreated seed in infested soil	Rice	64	3·0	11·1
	Pea	61	19·8	5·1
	Gram	85	12·6	8·0
Surface sterilised seed in sterilised soil	Rice	86	5·0	15·5
	Pea	87	25·5	6·8
	Gram	100	20·1	9·8

The data show that emergence in 14 percent rice seed did not take place on account of the organisms normally carried on unsterilised seed and 22 percent seed suffered from pre-emergence injury if unsterilised seed was sown in soil infested with *Fusarium sp.* When unsterilised pea and gram seeds were sown in infested soil, the pre-emergence injury was 26 and 15 percent, respectively.

(c) Beneficial effects of fungicidal seed dressings.

Fifty grams of pea (N. P. 29) and gram (N. P. 58) and 30 gm. of rice (N. P. (130) were treated with 12 fungicides for 15 minutes in separate Erlenmeyer flasks. Such treated and untreated seeds were plated on 2 percent potato dextrose agar pH 4·5). No fungi were obtained from rice, pea and gram seed treated with Agrosan GN, Agrosan G and Agrano, Lunasan, Granosan and Uspulun-Universal were equally effective in preventing growth of any fungi from treated seed of rice and pea but were not found effective enough for gram seed. No organisms were obtained from rice seeds treated with Mersolite, from pea and gram seeds treated with Ceresan and Spergon and from gram seeds treated with Hortosan A. Treatments with Ceresan, Hortosan A, Spergon, Semenon and Copper carbonate were ineffective against rice ; Semenon, Hortosan A and Copper carbonate were ineffective for pea. Lunasan, Granosan, Semenon, Uspulun and carbonate were ineffective against gram. In all these cases, a few colonies of *Fusarium sp. Helminthosporium sp.* and *Alternaria sp.* and other moulds were isolated from the treated seeds.

(d) Relative efficacy of different fungicides in green house tests.

In another series of experiments the untreated seeds and the fungicide treated seeds were sown in 5 rows, 3 inches apart in window pane boxes of size 20×16×4 inches which were filled with sieved field soil. Final emergence counts were taken on the twelvth day. After a few days, 50 seedlings of rice, N. P. 130 and gram N. P. 58 and 30 seedlings of pea N. P. 29 were selected at random. They were washed free of soil and dried between folds of blotting paper for determining the height and weight of seedlings, indicating the vigour of seedlings. The experiment was repeated thrice. Consolidated data are presented in Table 2.

TABLE 2

Relative efficacy of different fungicides on the emergence and vigour of rice, pea and gram seedlings

Treatments		Percent emergence	Weight of seedlings in gm.	Height of seedling in cm.
Agrosan G. N. (Ethyl mercuric chloride and tolylmercuric acetate 3 : 1000	Rice	90	5·7	14·6
	Pea	92	38·7	9·7
	Gram	96	39·8	10·3
Agrosan G. (Ethyl-mercuric chloride) 3 : 1000	Rice	88	5·1	13·4
	Pea	88	35·3	8·5
	Gram	93	34·2	9·7
Agrano (Ethyl-propyl mercuric bromide) 3 : 1000	Rice	77	4·7	11·2
	Pea	86	34·3	8·3
	Gram	83	33·1	9·5
Ceresan (Ethyl-mercuric chloride) 3 : 1000	Rice	88	5·4	12·6
	Pea	95	37·5	9·9
	Gram	87	32·0	8·8
Copper carbonate 3 : 1000	Rice	67	3·7	9·7
	Pea	68	26·2	6·7
	Gram	74	27·6	7·5
Granosan (Ethyl-mercuric chloride) 3 : 1000	Rice	66	4·1	9·5
	Pea	75	31·4	7·6
	Gram	80	31·1	8·6
Hortosan A (A nitro phenol mercury compound) 5 : 1000	Rice	72	4·3	11·0
	Pea	79	31·4	8·8
	Gram	79	30·2	8·5
Lunasan (Erhyl mercury thiourea) 3 : 1000	Rice	75	4·4	10·8
	Pea	78	30·1	7·8
	Gram	82	31·2	8·7
Semenon (A Phynl and organo mercury preparation) 3 : 1000	Rice	75	4·3	10·5
	Pea	76	32·0	7·1
	Gram	82	30·5	8·5

Treatment		Percent emergence	Weight of seedlings in gm.	Height of seedlings in cm.
Spergon (Tetrachloro para benzoquinone) 3 : 1000	Rice	75	4·3	11·0
	Pea	76	31·5	7·9
	Gram	80	34·9	9·0
Uspulun (A mercuric chlorophenol compound) 4 : 1000	Rice	86	5·2	13·3
	Pea	87	34·7	8·5
	Gram	86	32·4	9·4
Control	Rice	60	3·2	8·5
	Pea	60	18·4	6·4
	Gram	63	22·1	6·5

The data show that all fungicides were effective in stimulating better emergence, weight and height of seedlings. But appreciable increase in emergence, weight and height of rice and gram seedlings was obtained when Agrosan GN treated seed was sown. Pea seeds treated with Ceresan gave a better response as compared to other fungicides.

Dosage response tests carried out with 1·0, 0·5, 0·3, 0·1, 0·05, 0·02 and 0·01 percent Agrosan GN and Ceresan showed that the best emergence and vigour of seedlings was obtained when seeds were sown after treatment with 0·3 percent of these fungicides by weight.

IV. DISCUSSION

Muskett (1950) recently emphasised the significance of seed-brone fungi and the necessity of conducting studies of the type reported in this paper. Gopal Swarup (1950) studied the fungal flora of stored wheat, oat and maize, its relation with pre-emergence injuries and control by fungicidal seed dressings. These studies were extended to sorghum and barley by Murthy (1951) and rice, pea and gram seed by Sreekantiah (1952). Many fungi are reported to be associated with paddy in India and other rice growing foreign countries. In our samples, *Fusarium sp.*, *Alternaria sp.* and *Helminthosporium sp.* were mostly associated. Treatment with Agrosan GN not only destroyed these fungi but also stimulated better emergence and vigour of seedlings. Padwick (1938) reported the association of a number of fungi with pea seed in England and reported the efficacy of Ceresan in preventing seed rot caused by the associated fungi. *Fusarium sp.* was associated with the pea seed in our samples for which Ceresan treatment was most effective. (Delcanizo (1927) in Italy recommended the disinfection of gram seed by immersion in 0·5 percent Copper sulphate solution for 5 minutes against *Phyllosticta* (*Ascochyta rabei*). *Alternaria sp.* was mostly associated with gram seed used in the present investigation and treatment with Agrosan GN was found most efficaceous, Published literature as well as these studies show that seed dressings with fungicides not only inhibits the growth of associated saprophytic and pathogenic organisms and thus prevents pre-emergence injury, but also improves emergence and vigour of seedlings.

V. SUMMARY

The fungi associated with the rice, pea and gram seeds were isolated by the centrifugal and plate methods and identified upto the genus.

Fusarium sp. was predominant on rice. *Alternaria, sp., Helminthosporium sp. Penicillium sp.* and *Rhizopus sp.* were less common. *Fusarium sp.* was also predominantly associated with pea seed. *Alternaria sp., Aspergillus sp., Penicillium sp.* and *Rhizopus sp.*, were also fairly common. From gram seed *Alternaria sp.* was most frequently isolated followed by *Rhizopus sp., Mycogone sp., Aspergillus sp.* and *Fusarium sp.*

Reduced germination was obtained when seeds were sown in the presence of extra inoculum of the predominantly associated fungi *viz. Fusarium spp.* for rice and pea *Alternaria sp.* for gram.

In a series of laboratory and green house tests with 12 fungicides, Agrosan GN proved most efficaceous in destroying the fungi carried with rice and gram seed and in improving the emergence, weight and height of seedlings. Similarly Ceresan proved most efficaceous for the treatment of pea seed.

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ON SOME FRESH WATER LEECHES FROM NAINI TAL DISTRICT (U.P.)

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Naini Tal district is in the north-western border of the Uttar Pradesh. The northern portion of the district is hilly and the southern portion consists of plain areas. In the hilly region a number of streams flow between the valleys. Besides streams there are a number of lakes also, on account of which this district is sometimes also called "District of Lakes". The prominent lakes in the district are—The Naini Tal lake (6,300 ft.), the Bhim Tal lake (4,450 ft.), the Malwa Tal lake (3,600 ft.), the Sat Tal lake (4,500 ft.), the Naukuchia Tal lake (4,200 ft.) and the Garud Tal lake (4,550 ft.).

Leeches belonging to the following families have been collected from the localities indicated in each case.

RHYNCHOBDELLAE

Family Glossiphonidae

ARHYNCHOBDELLAE

Family Erpobdellidae
Family Hirudidae

FAMILY GLOSSIPHONIDAE

Placobdella ceylanica, Harding, 1909.

Glossiphonia ceylanica, Harding, 1909, p. 233.

Glossiphonia ceylanica, Kaburaki, 1921, p. 661.

Placobdella ceylanica, Moore, 1924, p. 357, pl. XIX, fig. 7 and plate XXI, fig. 35.

The colour of the body is light green in the living condition, On preservation in alcohol the colour becomes pale or light yellow. All the specimens were collected from a fresh water pool in Naukuchia Tal. No host parasite relation was observed, as all the specimens were found in free living state. This is the first record of this species from Uttar Pradesh.

Hemiclepsis marginata, subspecies *marginata*, O. F. Mueller, 1774.

Hirudo marginata, O. F. Mueller, 1774, p. 45.

Glossiphonia marginata, Moquin-Tandon, 1846, p. 375, pl. XIV, figs. 10-20.

Hemiclepsis marginata (in India), Kaburaki, 1921, pp. 694-695.

This subspecies is widely distributed in Kumaon as has been reported by Moore and Harding (1927) also. The colour is green. The intensity and the

distribution of the pigment varies in different parts of the body and in different specimens.

Glossiphonia weberi, R. Blanchard, 1897.

Glossiphonia weberi, R. Blanchard, 1897 (*b*), p. 332 ; Kaburaki, 1921, p. 695, fig. 1 ; Moore, 1924, p. 351.

The colour of body is light brown. The median row of papillae on the dorsal side is highly developed and forms a median longitudnal raised portion. On this median ridge is a broken longitudnal line formed by rust coloured chromatophores. The pigment over the rest of the body is disposed in 10 to 12 longitudnal lines on each side of the median ridge.

There are three pairs of eyes. The most common position of the Eyes in the specimens from Naini Tal is the first pair of eyes lying in ring 5 ; the second and the third pairs in the rings 7 and 8 respectively. Other common position of the first pair of eye is in ring 6. It has been found out that male and female ducts open by a common pore at XI/XII in this species. This finding confirms the presumption of Moore (1924) regarding the position of gonopore in the specimens of this species from Naini Tal.

FAMILY ERPOBDELLIDAE

Barbronia weberi, Blanchard, 1897.

Dina weberi, R. Blanchard, 1897, pp. 353-355 (figs. annulation).

Herpobdella hexoculata, Kaburaki, 1921, pp. 703-704 (fig. annulation).

Dina weberi, Moore, 1924, pp. 368-370, fig.

Specimens of this species have been collected from the Naini Tal lake, the Bhim Tal lake and slow running streams in the different parts of Naini Tal district. Colour, which so far has not been recorded in the living condition, is orange. Bigger the size of specimen darker is the colour. Some of the specimens gorged with food are brown to chocolate in colour. Small and starved specimens are translucent and very light in colour. The cocoons are laid during the autmn. Each cocoon contains 3-4 embryos.

Herpobdelloidea lateroculata Kaburaki, 1921.

Herpobdelloidea lateroculata, Kaburaki, 1921, pp. 705-706, fig 4.

Herpobdelloidea lateroculata, Moore, 1924, pp. 370-371.

There are five pairs of eyes. The first pair is on the dorsum of segment IV. In some of the specimens there is an indication of the presence of the sixth pair of eyes. One eye of the sixth pair has been seen in a number of specimens. Sometimes clitellum extends over sixteen annuli, covering first annulus of somite XIII also. Colour of living examples is orange. Structure of the alimentary canal has been found much different from that described by Moore (1924, 1927). Pharynx extends from somite VII to somite XII. Crop extends from somite XIII to somite XVIII. The number of chambers in the crop varies greatly. A number of patterns in the crop have been noticed. In the most common pattern the anterior portion of crop is formed by a long chamber followed by three short chambers. There is a tendency of the anterior chambers to fuse, thus resulting in decrease in the number of crop chambers. The chambers are thick-walled and the canals in between the chambers are thin-walled. In most of the specimens the first three chambers are

thick-walled and the rest three are with comparatively thin walls. The lumen of the intestine is projected into a number of caeca.

This species has so far been reported from Bushampur, Sagar and Manipur (Kaburaki, 1921, Moore, 1924). The present collection was made at Sat Tal lake. Some of the specimens kept in laboratory were observed swallowing plant fibres. Vegetation like algal filaments has also been obtained from the alimentary canal of this leech. These observations suggest the herbivorous nature of this species.

A specimen found in the Bhim Tal lake resembles *Herpobdelloidea lateroculata* in all the diagnostic characters, but differs from other specimens in the following points :—

1. The anterior end is pointed, whereas in the typical examples of the species the anterior end of the body is broader.

2. The crop is straight, thick-walled tube extending over 35 annuli. There are no chambers in the crop. At the anterior and posterior ends the crop is narrow and in the middle it is wide. Intestine extends over nine annuli only, whereas in typical specimens it extends over 11-12 annuli. Rectum is a straight, thick-walled tube like the crop and is of uniform diameter and not dilated at the anterior end.

3. The maximum width of the body is near the somite XVIII *i. e.* in the posterior half of the body, whereas in other specimens maximum width of the body is at the caudal end of the clitellum.

Dinobdella ferox, Blanchard, 1896.

Trocheta subviridis, Murie, 1865, pp. 659-662.

Whitmania ferox, Blanchard, 1896, pp. 322-325.

Haemopis birmanica, Kaburaki, 1921 (in part), pp. 712-713.

Whitmania ferox, Moore, 1924, 377-380, pl. XX, figs. 12-14.

Two sizes of leeches belonging to this species have been found. The first small ones, with empty alimentary canal are found in large numbers in the drinking pools of fresh-water streams. Their average length is 10 mm. in the state of fair extension. These are very agile. Body is dorsoventrally compressed. They are seen swimming actively. The other type comprises the large sized leeches. Their alimentary canal is gorged with blood. They are either found in the nasopharynx of cattle or in the water pools, where they lie inactive below the mud. Average size of these leeches is 80 mm. *i.e.* eight times the length of free living forms. In tranverse section their body is oval. A well fed specimen of this type measures in mm. length of the body 82 ; maximum width of the body 7 ; length of the anterior sucker 1 ; width of the anterior sucker 2 ; buccal width 0·4 ; length of the posterior sucker 16 ; width of the posterior sucker 15 ; distance from anterior end of body to the male pore 27.

The colour of the body is not green as reported by Moore (1924). The colour of small forms is slaty brown. A dorsal median field of lighter colour is also seen. The large well fed specimens are uniformly chocolate in colour with a dorsal median field of light chocolate colour. The segmental receptor organs are quite obscure.

The reproductive organs are small. These are particularly much reduced in the leeches which have just sucked blood *i.e.* in the parasitic phase. Epididymes are smaller and not much convoluted in the parasitic phase. In free living, small forms the convolutions of the epididymis form globular structures. Ductus ejaculatorius is longer in free living, small forms. Before entering the prostate this duct forms a loop. Atrium is not slender and elongated as described by Moore (1924). In parasitic forms the prostate is small and slightly bent over the penis sac. Penis sac is also small. Vagina is also not a slender structure as reported by Moore (1924).

Leeches of this species are widely distributed in Kumaon Hills and are probably the worst pest of the cattle in this area.

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LAND UTILIZATION PROBLEM IN ARID ZONE AND ITS SIGNIFICANCE IN SOIL CONSERVATION

By

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INTRODUCTION

It is well known that advanced civilisations formerly occupied what are to day arid regions. Excavations show remains of reservoirs, canals, fortifications and human habitations indicating an intensive and extensive cultivation by a large population. Such is the case in India, Pakistan, North Africa, the Near East and even in the desolate region of Lob Nor in Central Asia. There are remains of oasis towns and irrigation works. The disappearance of such civilizations may be attributed to actions of man, though not to the benefit of mankind. It certainly cannot be ascribed to severe climatic changes as is often thought to be. There is little evidence to show marked climatic changes since man used or misused land for living. The effectiveness of rainfall has, however, been seriously impaired by man's over use of the plough and the axe and by the grazings of animals, particularly the goats. Marginal areas have become man made deserts and in this sense, the desert areas are increasing. Using land beyond its capabilities, especially in the arid areas, upsets the balance between land, water and people which is more critical in arid areas than in any other climatic regions. When this balance is lost, the consequential effects are generally more severe than for humid areas, because recovery from the effects of over use takes longer time in arid zones than in the humid.

In any attempt to re-establish marginal areas for better production or habitation, it is necessary to survey and map the existing plant cover, both natural or cultivated and also land use.

For the purpose of proper land use in America, soils are grouped in eight capability classes. This produces an especially effective approach in arid and semi arid areas where land conditions and productions are so sensitive to land use.

These classes range from class I—the best kind of farm land to class VIII, characterised with such severe limitations that it cannot be used for forestry grazing or cultivated crop production.

The soil classes on the whole, fall into the general groups—those that are suitable for cultivation and those which are unsuitable. Each class is distinguishable from the others by the relations of definite physical features to the intensity of using wood lands, cultivation, pasture range, and wild life, and the intensity of soil management required for safe and sustained use. Class I, II, III and IV should be restricted to limited cultivation, while classes V, VI, VII and VIII are

*Now known as Resource Utilization Studies Division of the Central Arid Zone Research Institute.

suited for grazing, wood lands, wild life and recreational purposes in descending order of intensity of use.

The principle of capability use when violated results in low financial returns and acceleration of soil erosion. In the end it may mean permanent soil loss that reduces productivity and the land might have to be abandoned. The phenomenon of land abandonment is a common spectacle in arid areas which signifies land use failure.

Erosion Damage :

It is a common sight in Western Rajasthan, that lands are being used beyond their capability, which are, thus deteriorating more rapidly than in other parts. Here the annual precipitation is low and lands used beyond their capability become sandy or shallow phased. Under such conditions, the production of vegetative cover is frequently inadequate to keep the soil intact and as such both wind and water erosion take heavy toll. Such a damage would be destructive to any class of soil but the damage caused to shallow phased soils is more likely to be irrepairable than in case of deeper and better grades of lands. And yet, because in wet cycle, lands often produce high yeilds, there is a strong tendency to gamble on them year after year. This results in blowing of soil from the fields and formation of sandy wastes and sand dunes, thus, turning the land infertile and useless.

In the arid lands of Rajasthan, it has been found that out of a total utilizable surface and ground water potential of 12 million acre feet only about 4·3 million acre feet have been utilized in constructing "Nadis", "Tanks", "Ponds" etc. (Kanwar Sain, 1956), in descending order of their frequency. Thus, there is a great scope for further utilization of ground water resources for adopting various water conservation practices.

Low vigour is exhibited by the variegated appearance of the leaves of plants. This variation emphasises a sharp change in the amount of nitrogen and other micro-nutrients available. Experiments in a 90 mile desert of Australia have shown that additions of 7 lbs. each of cobalt and manganese per acre prove very effective in increasing crop production. This unnecessary fertility depletion is more serious in arid areas, because of our ignorance to utilize commercial fertilizers effectively, particularly in areas having low rainfall. The problem of rebuilding in eroded and depleted soil is further complicated by limited number of adaptable legumes and other plant species suitable for inclusion in the cropping systems or afforestation programme in arid areas.

Role of Conservation Practices in Arid Zone :

The use of appropriate conservation practices will slow down the process of land damage even when the land is being used for purposes for which it is not suitable. However, the cost of applying the measures that will safeguard lands under such misuse is too high and soon reaches the point of a diminishing return, especially when the entire area is being used beyond the capability of its soils. Here the conservation programme is largely a delaying action that takes time in which to get the land used in harmony with its physical, climatic and economical limitations. In this case, where suitable conservation measures are not employed, the rapid deterioration of land makes limited selection much more difficult or even virtually impossible. Wherever soils are suitable and adequate water supply is available, irrigation is an effective means of stabilising farming in arid areas ;

Ganganagar in Bikaner division (Western Rajasthan) provides an example of the value of this type of land use. Here 4 million acres have been brought under irrigation. Some caution is required, especially, where underground water is used. In many areas these supplies are already being depleted faster than they are being replenished.

For a successful farming without initiation in arid and semi arid lands of Western Rajasthan, the farmers must accept highly variable and fluctuating rainfall as a normal phenomenon. Once these farmers undestand this as a cropping limitations, they can develop their farm enterprise in harmony with the pattern. *This means gearing the farm programme to the dry phase of weather cycle rather than to the wet periods.* The first case is, therefore, an approach to successful farming because it reorganizes the capability of land.

The present picture of land use in Rajasthan indicates the consequences of using arid and semi arid lands beyond their capability and of inducing aridity in regions not actually arid climatically. The task before the Desert Afforestation and Soil Conservation Station is to explore the possibilities for increasing and maintaining the sustained production from forests, cultivated fields and grasslands in arid zone without imparing soil fertility. The Station has been set up at Jodhpur and work is in progress in the three major land uses *viz.* forestry, agriculture and agrostology.

Silviculture :

The forester also has an important role to play in this arid and semi arid region. Cutting of relict tree and shrub vegetation for fuel and fodder leads to wide spread destruction of plant cover consequently resulting in ecological regression. There is great scope for planting fuel lots, wind breaks and shade avenues, to provide protection to crop areas or livestock concentrations and fodder trees for supplementary feeds during famine periods. The graziers and cultivators must also realise the ameliorating influence of forests stand on extremes of climate and in relation to soil and water conservation. The forester is still losing ground rapidly on account of encroachment of grazing in forest areas, the ruthless cutting of trees for fuel or building purposes and insatiable demand for land for the production of food and cash crops. An intelligent plan of land utilization based on land capability classification must allow for an adequate percentage of forest cover in the right place which must be composed of the most desirable species for meeting the needs of local population.

Arid tracts are generally characterised by scarce and eratic rainfall, poor and degraded soils and badly wind eroded lands. It is, therefore, imperative that before reclaiming the areas, techniques be developed taking into consideration all the locality factors. It is the degree of uniformity and conformity between environments which is of great help in drawing sound parallels for the development of vegetation on such arid and semi arid lands. Determination and significance of the natural succession and the stage at which the more exacting species should be introduced, greatly aids in the planning of afforestation programme.

The main Silvicultural problems in the arid and semi arid areas are : (1) Selection of suitable species adopted to varied physical environments, (2) the different afforestation techniques and (3) the management of afforested areas consistent with the needs of the soil and the man.

The main task, therefore, for the Silviculturists is to attempt regeneration of a better plant cover and to do this while the population is engaged in growing a

living from the area. To study such natural regeneration as may occur, it is essential to have a guide in this work, enclosed or protected areas which are ungrazed. Sometimes the results may be startling. One thing of course, cannot be done and that is to go over from one area in one part of the world to another area in another part and at once begin to apply the procedure in the expectation that it will be successful. *Much is needed to select suitable plants, to get them to seed, to germinate and to grow in those very old environments.*

From Silviculture stand points, the main objective in pushing back the desert from this region in which aridity is primarily man made and limiting it to the area with a true desert climate will be the regeneration of vegetation by natural and artificial means. This work must be carried out in regions with ancient forms of land use and against a back-grounds of increasing human and livestock population which must continue to obtain their livelihood and sustenance from the land while the improvement is going on. *If the biotic factors could be limited for some five or ten years, it would be possible to make full use of the remarkable capacity of degraded types of vegetation to revive and climb up the ecological ladder to provide a superior type of vegetation.* It would then be possible to evolve new control system of land use to provide better resources and types of food, fodder or fuel without again causing the degeneration of plant cover back to its present conditions.

The Silvicultural section of the Desert Afforestation and Soil Conservation Station, Jodhpur is at the present working on the following problems : (1) Selection of species to such varied physical environment, (2) Growing of seedlings in a manner to endure adverse conditions, (3) Development of suituble techniques for sowing and planting, (4) Moisture conservation and economic utilization of available water, (5) Optimal time of planting, (6) Nutritional requirement of indigeneous and exotic species both with respect to major and micro-nutrients, (7) Movement of sand dunes, their stabilization and afforestation, (8) Most suitable species and design for wind break and shelter-belts, and (9) Optimal lopping regime for important fodder tree and shrub species etc. (Kaul—1958). It is hoped that in the near future, important silvicultural information on various tree and shrub species of the region, which have hitherto remained completely un-investigated will be available. This information will not only be helpful to the foresters of Rajasthan but will also be of great use to the forester in other arid and semi arid regions of the country.

Arostolggy :

According to the 1956 livestock census, Rajasthan harbours about 12 million cattle, 8·7 million goats and about 7·3 million sheep against the all India figures of 155, 47·1 and 38·2 millions respectively. The cattle population of Rajasthan approximates 8%, sheep and goat about 20% of the total Indian figure. Forty percent of the total wool production of the country is contributed by Rajasthan alone and as such its place regarding sheep industry, in the welfare of the State, ranks high.

The entire livestock population of the whole State and more particularly of the Western Rajasthan, mainly subsists on native grasslands. It would, therefore, be seen that the first step in building up of a organized sheep or cattle industry would lie in the development of grassland resources of the State. This question becomes all the more important for Rajasthan where the fodder resources owing to continued over and misuse have contributed conspicuously to desert formation.

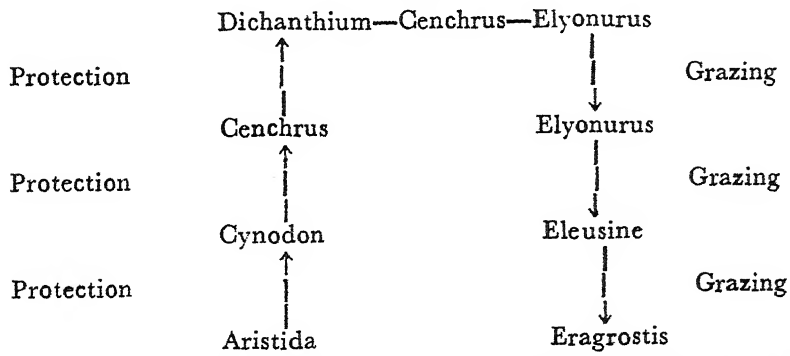
According to even rough estimates, a minimum of 5 to 7 acres of grassland, maintained in excellent condition, would be needed for a cow unit to subsist

entirely on these resources. In general, the grasslands in this region are very poor and as such 15-20 acres are needed to maintain a cow unit in normal health. The present rate of grazing incidence in all the districts, therefore, varies from at least 2 to 6 times more than the grassland condition can sustain. Areas which are away from the villages and watering points, are genereally not so over grazed and the chief grasses found grown in these areas are *Elionurus hirsutus*, *Cenchrus ciliaris*, *Cenchrus setigerus* and *Panicum antidotale.* The grazing intensity becomes magnified owing to non-availability of water in most of these areas. Animal activity is, however, restricted round about water sources which leads to overgrazing of certain areas, resulting in regression. The vegetation in such areas consists of few annual species *viz.*, *Aristida funiculata, Aristida adscensoionis*, *Cenchrus cathartieus. Tragus biflours*, *Latipus senegalensis* and *Indigofera cordifolia.* The vegetation on must of the native grasslands have, thus, degenerated from a good pasture type dominated by perennial grasses to a poor or very poor type dominated by annuals and other useless legume species as shown in the following diagram (Dabadghao—1957).

Dichanthium—Cenchrus—Elionurus type

Progressive & regressive

Stages in



It is, therefore, clear that the vegetation could reach the climax type again if protection to these grasslands is afforded and thereafter they are managed under some system of deferred grazing.

Keeping the above points in view, the programme of Agrostological research has been organised under two major heads *viz.*, pertaining to cultivated and natural grasslands respectively. The activities of this section have been grouped under four broad categories *viz.* (1) Grass legume nursery studies, (2) Agronomic studies, (3) Grazing studies ; and (4) Ecological studies.

The results of the above mentioned investigations will forms the basis of developing grassland economy of this region.

Agronomy :

As a result of improper agronomic practices, the problem of wind erosion has accelerated. particularly in the arid parts of Rajasthan, where only 31·35% of the land is under cultivation and the bulk of which is under dry land farming. The reclamation of desert will bring in its wake the problem of raising crop yields on a sustained basis without impairing the soil fertility and to utilise the available land economically. Research in agronomy has to be aided by studies on crop rotations,

methods of tillage, nuturitional requirements, improved varieties and soil and moisture conservation practices (Misra—1958). Results from such studies will be helpful in better land use planning. For this purpose the Agronomy section of the Desert Afforestation and Soil Conservation Station is carrying out research on (1) Crop introduction and varietal trials, (2) Cultural manurial and rotational trials on major crops of arid zones *viz.* Bajra (*Pennisetum typhoideum*) Moth (*Phaseolus aconitifolius*), Moong (*Phaseolus radiatus*), Guar (*Cyamposis psorialioides*, (3) Wind strip cropping and Stubble mulch farming for wind erosion control, (4) Drought resistance studies in crop plants, (5) Studies on wind break and crop yields etc.

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ON THE COMMON HELMINTHIC AFFECTIONS OF THE SMALL INTESTINE IN INDIAN SHEEP

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Amongst the large number of helminths parasitising the small intestine of sheep, the species in Indian sheep are recorded in the compilations/reports of Bhalerao (1935, 1942, 1942*a*), Baylis (1936), Sarwar (1945), Thapar (1956), Varma (1957), Gupta (1958) and Yamaguti (1958). Some of these parasites, particularly in heavy infestations of the duodenal region, are associated with prominent disorders which may often lead to high degree of mortality specially amongst the young stock probably from the many histopathological changes in this important part which is concerned, not only with the digestion of proteins, carbohydrates and fats, but with the partial absorptive work of some of these products of digestion as well.

The helminthic diseases of sheep, specifically recorded so far in our country, are immature paramphistomiasis reported by Haji (1935), Srivastava (1938, 1947), Bawa (1939), Bhalerao (1944), Kuppuswamy (1948), Iyer (1949), D'Souza (1949) while Pande (1935) and Mudaliar (1945) described similar infection for cattle and goat ; monieziasis. ascariasis and strongylosis—all listed by Rahim-ud-Din (1937), and cymbiformiasis by Katiyar (1956). From other countries, trichostrongylosis (black scour disease), cooperiasis, nematodiriasis, ancylostomiasis (hookworm disease), strongyloidosis and cestodiasis have additionally been mentioned.

The collection of helminths and the diverse types of infested material, made at the slaughter houses at Mathura, Nainital and Almora, from this part of the alimentary tract yielded *Trichostrongylus colubriformis* (Giles, 1892) Ransom, (1911), *Cooperia punctata* (v. Linstow, 1907), *Bunostomum trigonocephalum* (Rudolphi, 1808) Railliet, (1902,) *Gaigeria pachyscelis* Railliet and Henry, (1910), *Strongyloides papillosus* (Wedl, 1856) Ransom, (1911) and *Nematodirus filliocolis* (Rudolphi, 1809) Ransom, (1907) amongst the nematode ; *Moniezia expansa* (Rudolphi, 1810), *M. benedeni* (Moniez, 1879), *Avitellina* spp. and *Stilesia* spp. amongst the cestode and *Ogmocotyle indica* (Bhalerao, 1942) Ruiz, 1946 (Syn. *Cymbiforma indica* Bhalerao, 1942) and a variety of immature amphistomes amongst trematode parasites. It has been possible to undertake an assessment of the pathogenic effects exhibited in the naturally infested material from microscopic study of the serially cut and stained sections. As a rule, multiple infections are frequent and an average worker, unless familiar with the various lesions, their histopathology around the stages of the specific parasites harboured and the nature of pathogenicity, is likely to err in the diagnostic work—an important prerequisite for treatment and control. A brief account of such data has, therefore, been attempted.

1. Trichostrongylus colubriformis.

According to Lapage (1956) *Trichostrongylus* spp. affect sheep and goats more severly than cattle, the symptoms of trichostrongylosis, as mentioned by Newsom

(1952), being diarrhoea, loss of appetite, emaciation and presence of bottle-jaw. Lapage (1956*a*) also believed that the worms usually do not cause oedematous swelling and the mortalities, when occurring, were from the toxic substances elaborated by them. The clinical picture and the pathological changes in enteritis, caused in *T. colubriformis* infection in lambs, have been described by Kates and Turner (1953).

This widely distributed species was encountered in nearly all the sheep examined locally but with a varying intensity and in the Tibetan and Hill sheep the infection was occasional. It occurred always mixed with other helminths and in heavy infestations the mucosa exhibited copious amount of thick opaque mucus with a few haemorrhagic streaks. The worms, both adult and juvenile forms, could be detected in the sediment after thorough washings of the contents and scrapings of this part. The histological examination of the lesions revealed the different stages embedded inside the mucosal tunnels which at times had extended upto the muscularis mucose (Plate I, Fig. 1). A large number of worms occurred free in the lumen or between the folds of the mucosa or even on the mucosal surface but were always covered with a thick layer of mucoid material which inculded masses of cellular debris. The tunelling had resulted in desquamation and erosion of the surface epithelium, destruction of the glandular part of the mucosa, congestion of the sub-mucosal blood vessels and an ulceration of the intestinal wall probably from secondary infections. The mucus glands also revealed some degree of degenerative changes, a cellular infiltration mainly with polymorphonuclear leucocytes and the tunnels, left by the wandering females, contained characteristically segmented eggs.

2. Cooperia punctata.

The species, *C. cuticei*, in sheep is believed to be a comparatively non-pathogenic nematode. Andrews (1939), giving an account of its pathogenesis in an experimental infection, found that around the worms the intestinal mucosa had developed nodules which was believed to be an act of resistance against superinfection. Light infections with *Cooperia* spp., according to Lapage (1956*a*), were of no consequence but in heavy infections young sheep showed symptoms similar to those of trichostrongylosis but with a more marked anaemic condition.

Lapage (1956*a*) thought this, being a common parasite of cattle, accurs rarely in sheep. It was recorded from cattle in Madras by Rao (1940), from goat at Mukteswar by Bhalerao (1942) and was collected only once which thus constitutes its first record in Indian sheep. A large number of worms were found in the anterior region of duodenum in one of the Hill sheep examined at Almora.

Histological study of the sectioned material revealed a few of the worms that had penetrated into the mucosa or were found lying coiled deeply inside it. Some of the worms were also present superficially on the mucosa or free in the lumen. In the sections, the mucosa exhibited minute but somewhat prominent nodules bearing a centrally necrosed area which was surrounded by fibroblasts and a leucocytic infiltration. No stage of the parasite, however, was harboured.

This naturally infested material appeared to have carried a comparatively light infection or one that had been of some standing because nodules were present at places.

Fig. 1. Photomicrograph of a section showing an adult female of *T. colubriformis* penetrating the mucosa (note the characteristic feature of the genitalia). 60 x.

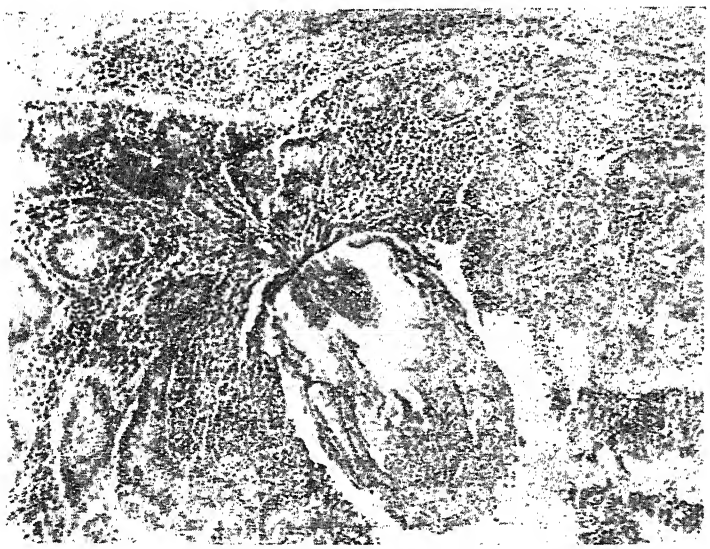

Fig. 2. Photomicrograph of a section showing the large buccal capsule of *G. paclyscellis* with a plug of mucosa and the prominent sub-mucosal lancet inserted deep inside the patch. 130 x.

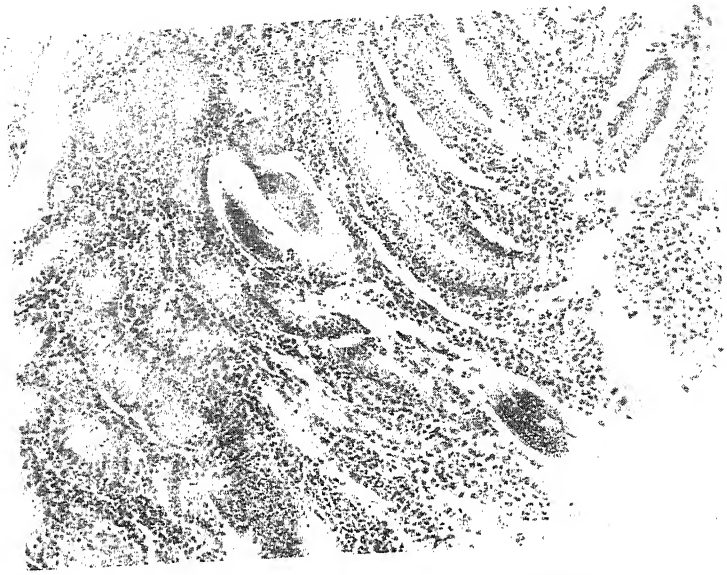

Fig 1. Photomicrograph of section showing the parasitic female of S. *papilloisus* embedded inside the mucosa. 140 x.

Fig. 2. Photomicrograph of a section showing the suckers of an immature anoplocephalid tapeworm with the tucked-in mucosal patch. 110 x.

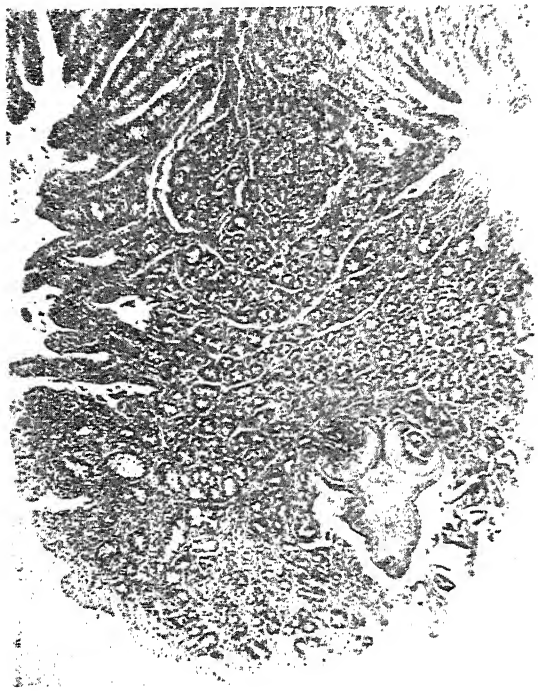

Fig. 1. Photomicrograph of a section showing a nodule-like raised area with the centrally embedded scolex of an immature tapeworm 35 x.

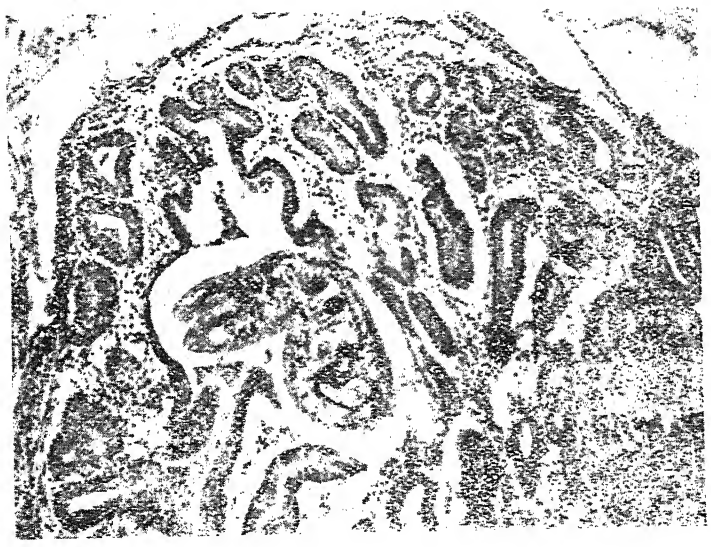

Fig. 2. Photomicrograph of a section showing an immature amphistome lying embedded near the sub-mucous region. 90 x.

3. Hookworms (Bunostomum trigonocephalum and Gaigeria pachyscellis).

Hookworms, being voracious blood-suckers, have a great significance as cause of anaemia in ruminants and those recorded from Indian sheep belong to three species of which *B. trigonocephalum* and *G. pachyscelis* were collected from the duodenum and jejunum in both the plain and hill regions. *G. pachyscelis*, found in the local sheep, appears to be more common than *B. trigonocephalum*. In consequence of the firm attachment effected by the worm through its capacious buccal capsule and the powerful oesophagus, prominent haemorrhagic spots are distinctly seen on the mucous membrane. At the older sites, however, the points of feeding can be identified as black clots.

From the histological examination of the serially cut sections in *G. pachyscelis* infection, the extent of the injury inflicted by a worm at the point of attachment could be studied and in heavy infestations a greater area of the mucosa appeared involved. The buccal cavity, deeply embedded as far the muscularis mucosae, contained a strong mucosal plug with the prominent subventral lancets partly piercing the patch (Plate I, Fig. 2). From necrosis of the plug and the subsequent development of a scar tissue, as evident in some of the sections, severe mechanical damage was evident in the wall which also exhibited a prominent increase and congestion of the blood-vessels with profuse haemorrhage in the neighbourhood. The affected region throughout showed a marked development of the lymphoid tissue, a heavy eosinophilic infiltration and an excessive amount of mucus in the gland cells.

4. Strongyloides papillosus.

Turner (1955), studied the experimental strongyloidosis in lambs that had died and described the pathological changes mostly produced in the duodenum and to some extent in jejunum. Later Turner and Wilson (1958), during an outbreak of the disease in lambs, reported a few deaths on pasture and Garkavi (1956), after describing the lesions and symptoms of disease, concluded that this species, pathogenic in young lambs, produced acute symptoms and death.

The species, first recorded from cattle amongst the Indian domestic ruminants by Vaidyanathan (1942) and subsequently by Sarwar (1945) in sheep and goats, was encountered both in the local and the Hill sheep.

The pathogenic effects are produced by the parasitic females which lie embedded in the mucosa. The histological study of the material collected from the Hill sheep, harbouring the infection singly or mixed with the ovine monostome, provided details for a comparative assessment of the nature of histopathology in this specific infection.

The worms, found embedded inside the villi or penetrated deeply in the glandular part of the mucosa (Plate II, Fig. 1), had left characteristic strings of eggs, either segmenting or with a developed embryo, inside the tunnels. Erosion, damage to the gland cells, infiltration with the lymphocytes and eosinophiles with haemorrhage in the mucous membrane—suggestive of a catarrhal enteritis, were all evident.

5. Cestodes and Cestodiasis.

Mudaliar *et* Mudaliar (1942), while reporting "monieziasis" in lambs harbouring these worms stated that the coat was rough and there was falling off of the wool, greatly pendulous abdomen, wasting and greenish thin diarrhoea. It was also

observed by them that in tapeworm infestations a great mucosal pallor of the type seen in animals that had been bled to death and not much of enteritis were other features which were explained as due to a loss of the nutrients and a large depletion of blood calcium. Newsom (1952), citing Ward and Scales (1946), Hebermann and Carlson (1946) and Tabelman (1946), held that a heavy tepeworm infestation was associated with diarrhoea, loss of weight, emaciation and even acute convulsions ending in death. There are, on the other hand, the views of others like Hawkins (1946) and Kates and Goldberg (1949) who regarded that these tapeworms were either non-pathogenic or exhibited a mild degree of pathogenicity. Lapage (1956) believed that the nature of the harm done was not fully understood and infestation deprived the host of certain elements but, in a heavy infestation, the sucker would cause irritation of the bowel wall.

Mostly the adult tapeworms were found with their scolices superficially attached but in some cases a large number of immature forms were seen deeply embedded in the anterior region of the duodenal wall. Such sites exhibited a nodule-like raised area which, on teasing, revealed the centrally situated scolex and the neck portion. At times in massive infestation the immature stages were concentrated over a smaller area of the duodenal mucosa and in more advanced form such places caused a reduction of the duodenal lumen in consequence of the wall being packed up with masses of the developing forms which had copious mucoid exudate around. In some cases the embedded scolices were identified as those *Stilesia* spp.

In the serial sections the scolices, either attached superficially or embedded deeply, contained inside the powerful sucker mucosal plugs (Plate II, Fig. 2). The entire scolex was completely buried inside the mucosa (Plate III, Fig. 1) which showed degrees of degenerative changes consisting of necrosis in the superficial part, proliferative changes in the gland cells and the development of increased interglandular tissue which caused the formation of the raised area, invariably noticed around an embedded scolex. The extent of cellular infiltration depended on the extent of the tissue damage inflicted. Eosinophiles and lymphocytes, as usual, were observed at such sites.

6. Immature paramphistomiasis.

Heavy infestations of sheep with immature amphistomes have been reported from many countries of the world. Borary (1959) has reviewed the existing literature on intestinal amphistomosis including the work of the Indian authors.

Incidence of immature amphistoms in the initial part of the duodenum was mostly observed in the mid-winter season. The adult amphistomes, collected from the rumen from time to time, were *Paramphistomum cervi* (Zeder, 1790), *Cotylophoron cotylophorum* (Fischoeder, 1901) Stiles *et* Goldberger, 1910 and *Grastrothylax crumenifer* (Crepl., 1847) Otto, 1896. The immature specimens occurred firmly attached to the mucous membrane which showed a marked congestion and an increased mucoid secretion on its lining.

On histological study of the stained sections of such lesions, a large number of immature amphistomes of variable sizes were cut, some being attached to the mucosa with a part of the lining inside the powerful acetabulum while some others, particularly of a smaller size, had reached the deeper sites extending even upto the muscularis mucosae in the sub-mucous region (Plate III, Fig. 2). The maximum number found attached or embodded at one place numbered upto eight. A definite evidence of necrosis of the mucosa with a marked thickening of the intestinal wall was apparent. The supporting reticular connective tissue of the

glands had prominently increased and the blood-vessels were found congested. Evidence of haemaorrhage and a leucocytic infiltration was also noticed.

A study of these serial sections also revealed the presence of the eggs of *Schistosoma indicum* Montgomery, 1906 deposited at different depths of the mucosa. An evidence of haemorrhage with an eosinophilic infiltration was marked. This location of blood fluke eggs in the anterior region of the duodenum, herein reported for the first time in natural infestations, probably resulted from a heavy infection and in consequence of the adult worms having invaded the tributaries of the gastro-duodenal vein.

7. Ogmocotyle indica.

This monostome, with its boat-shaped or canoe-like body and margins well suited for facilitating its attachment, was incriminated by Katiyar (1956) with the disease, "cymbiformiasis" which cannot stand as a specific entity as reported by Pande *et* Bhatia, 1960.

CONCLUSION

The present study of naturally occurring infestations in seven different types of helminthic infections of duodenum has shown that *T. colubriformis*, *C. punctata* and *S. papillosus* could at times act as important pathogens particularly in young stock or in the imported breeds or their progeny from cross breeding or in cases of malnutrition or if and when subjected to adverse conditions from stress, strain or from harbouring of other infections. The pathological changes described around the embedded scolices of immature anoplocephalids, which support the views of Lapage in regared to the irritation in the intestinal lining, have also for the first time been recorded. The details about the infections in case of immature amphistomes help in presenting a connected picture of the nature of damage that can similarly result from the diverse pathogenic species that may affect the duodenum and in these cases the detection of the specific stages could alone help in a diagnosis of the associated disorders.

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NITROGEN REQUIREMENTS OF SOME MEMBERS OF THE FAMILY SAPROLEGNIACEAE*

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INTRODUCTION

Nitrogen plays a vital role in the nutrition of fungi. The information on this subject, though extensive, is far from being complete. It has been known that all nitrogen sources are not equally suitable for all fungi, which are very specific in their choice of this element. The investigations of several workers clearly show that closely allied species of the same genus may differ considerably in the choice of their nitrogen source.

The nitrogen requirements of saprolegniaceous fungi have been studied by Volkonsky (1933, 1934), Leonian and Lilly (1938), Saksena and Bhargava (1941), Bhargava (1945), Whiffen (1945), and Resichler (1951). Leonian and Lilly (1938) stated that five members of this family grew well on a medium in which ammonium nitrate and L-cystine were the only added nitrogenous compounds. Saksena and Bhargava (1941) and Bhargava (1945) found that ammonium nitrate and ammonium sulphate supported the best growth of five species belonging to the order Saprolegniales in the presence of sodium sulphide (or potassium sulphate) for (*Brevilegnia gracilis*) as a sulphur source. Whiffen (1945) however, reported that her isolates were unable to grow with ammonium sulphate as the nitrogen and cystine as the sulphur source (with the exception of *Dictyuchus monosporus*. All these above investigators agree that members of the Saprolegniaceae do not utilize nitrogen with the exception of *Brevilegnia gracilis* (Bhargava, 1945) and *Dictyuchus monosporus* (Whiffen, 1945) which grew poorly on it. They are also of the view that amino acids when used singly supported much better growth than any other nitrogenous compound. For some members of the family Saprolegniaceae. Volkonsky (1933, 1934) reported that alanine, serine, phenylalanine and cystine were superior amino acids while Bhargava (1945) and Whiffen (1945) came to the conclusion that glutamic acid was the preferred nitrogen source.

It has been observed by Resichler (1951) that members of the Saprolegniaceae do not require specific amino acids, but still do not seem to grow well or at all with ammonium salts as the only source of nitrogen. The present investigation was undertaken with the aim of adding to our knowledge the nitrogen needs of some members of the family Saprolegniaceae, viz., *Achlya aplanes*, *Isoachlya unispora*. *I. toruloides* and *Saprolegnia parasitica*.

MATERIALS AND METHODS

Various nitrogenous compounds (inorganic and organic) were added signly to the basal medium** in amounts calculated to furnish 700 mgm. of nitrogen per litre. Cystin and peptone were tried in 0·1% concentration because of the low solubility of cystin and unknown composition of peptone. The media were autoclaved at 15

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**KH_2PO_4–0·5 gm., $Mgcl_2.6H_2O$–0·5 gm., Na_2S–0·17 gm., dextrose 5·0 gm., and distilled water 1000 ml.

lbs. pressure for 15 minutes and the pH of the media was adjusted to 7·0 before autoclaving. Material for the inoculation was taken from the actively growing colony and the inoculations were made by agar disc method. The incubation period was 15 days and the experiment was run at 25°C (± 1°C).

TABLE 1

Showing the dry weight (in mgm.) of the four fungi on media containing equivalent quantities of different nitrogen compounds.

Nitrogen compounds.	FUNGI			
	Achlya aplanes	*Isoachlya unispora*	*Isoachlya toruloides*	*Saprolegnia parasitica*
Ammonium nitrate	14·7	19·7	18·0	21·0
Ammonium sulphate	6·0	15·0	11·0	7·0
Sodium nitrate	0·0	0·0	0·0	0·0
Potassium nitrate	0·0	0·0	0·0	0·0
Sodium nitrite	0·0	0·0	0·0	0·0
Glycine	13·7	16·3	6·4	6·0
dl–alanine	13·3	28·7	16·0	5·0
dl–valine	11·0	20·0	37·0	15·0
dl–leucine	4·3	12·0	23·0	33·0
dl–serine	20·0	6·7	6·6	3·0
dl–aspartic acid	21·0	28·0	21·0	36·0
dl–glutamic acid	69·0	58·7	68·0	61·4
dl–asparagine	12·7	24·7	27·7	14·0
l–arginine	19·0	25·0	22·0	40·0
l–histidine	26·0	38·0	53·0	57·0
l–cystine	11·4	29·7	22·6	23·3
Acetamide	34·0	19·0	19·0	25·0
Peptone	28·7	24·3	30·7	16·3
Control	0·0	0·0	0·0	0·0

EXPERIMENTAL

The various media were inoculated with the above mentioned organisms and the basal medium alone served as control. The results after statistical analysis are summarized in Table 1.

The data presented in Table 1 clearly show that different amino acids vary in their effectiveness and that different species of the same organism may respond differently to the same source of nitrogen. The two ammonium sources of nitrogen behaved differently for the fungi studied. Ammonium nitrate was found moderate while ammonium sulphate was poor for the growth of all the organisms excepting *I. unispora* which grew moderately on ammonium sulphate.

The two nitrates *viz.*, sodium and potassium nitrate and sodium nitrite proved valueless sources of nitrogen for the growth of all the four organisms.

All the five monoamino monocarboxylic acids exhibited different behaviour with the fungi under experiment. Glycine proved a mediocre source for the growth of *Achlya aplanes* and *I. unispora* but poor for rest of the fungi. Dl-alanine supported good growth of *I. unispora*, but moderate of *Achlya aplanes*, *I. toruloides* and poor of *Saprolegnia parasitica*. Dl-valine was good for the growth of *I. toruloides* but moderate for *I. unispora*, *Saprolegnia parasitica* and poor for *Achlya aplanes*. Leucine proved to be a good source of nitrogen for the growth of *Saprolegnia parasitica*, mediocre for *I. toruloides* and poor for *A. aplanes* and *I. unispora*. Serine gave good growth for *A. aplanes* but was a poor source for the rest of the fungi.

Both the dicarboxylic acids, *viz.*, aspartic acid and glutamic acid were found to be good sources of nitrogen for all the organisms except *I. toruloides* for which aspartic acid only was a moderate source.

Asparagine proved to be a good nitrogen source for the growth of *I. toruloides* but only moderate for the rest of the organisms.

Arginine was able to support good growth of *S. parasitica* only and fair in the rest of the fungi. Histidine proved to be a good source of nitrogen for all the four fungi.

Cystin supported good growth of *I. unispora* mediocre of *J. toruloides* and *S. parasitica* and poor of *A. aplanes*.

Acetamide was able to give good growth of *A. aplanes* but only moderate for the rest of the fungi.

Peptone was good source for the growth of *A. aplanes* and *I. toruloides* and moderate for *I. unispora* and *S. parasitica*.

None of the organisms under study were able to grow in the medium devoid of any nitrogen source.

DISCUSSION

From the results obtained in the present investigations, it is evident that *Achlya aplanes*, *Isoachlya unispora*, *I. toruloides* and *Saprolegnia parasitica* are unable to utilize nitrates or nitrites but give good response to ammonium as well as organic nitrogen. Therefore, they very well fit in the third group, "Ammonium organisms" of the Robbins classification. According to Lilly and Barnett (1951) a fungus which utilizes nitrate nitrogen must be able to reduce it to the oxidation level of ammonia. Failure of fungus to utilize nitrate nitrogen is coupled with inability to perform this reduction. It appears to the author that in the present investigations the failure of the organisms to grow on sodium and potassium nitrate is due to their inability to perform the necessary reduction as suggested above.

It has now been proved by many workers that nitrites are toxic for the growth of many fungi. Lilly and Barnett (1951) are also of the opinion that they have a destructive effect on proteins and amino acids and therefore they are rarely used in the preparation of a medium. The fungi tested by the author failed to utilize sodium nitrite and are in agreement with the findings of Leonian and Lilly (1938) and Bhargava, (1945).

Of the two ammonium salts used in the present studies, ammonium nitrate was found to be a moderate source for the growth of all the organisms while ammonium sulphate was a poor source. When a fungus is grown with an ammonium salt as the sole source of nitrogen, the reaction of the medium tends to become acidic. Fungi vary in rate at which their growth leads to increasing acidity of an ammonium salts medium and also in their toleration of a low pH.

All the five monoamino monocarboxylic acids, *viz.*, glycine, alanine, valine, leucine and serine supported varying growth of the fungi under experiment. Here we find that a given amino acid allows very good growth of one organism and only very little growth of another. Hence it is very difficult to give any adequate explanation for this as whether this reflects permeability, enzymatic capacities or merely such secondary problems as acidity changes consequently upon utilization.

Both aspartic acid and glutamic acid proved good sources of nitrogen for the growth of all the fungi studied except *I. toruloides* for which aspartic acid only was a moderate source. Of the two monoamino dicarboxylic acids glutamic acid proved to be more favourable. The good growth on glutamic acid can be attributed as explained by Waksman and Lomanitz (1925) to (*i*) much larger ratio of the carbon to the nitrogen and that (*ii*) glutamic acid being very favourable for respiration, resulting in the formation of very little volatile acids. This is further explained by the fact that when the ratio between carbon and nitrogen is high, the amount of ammonia produced will be less, because the fungus will continue to grow and derive its energy from the available carbohydrate and the ammonia which is a waste product in carbon metabolism will be utilized resulting in greater amount of growth.

Compounds which readily yield ammonia, such as asparagine, have been reported as excellent sources of nitrogen by Brock (1951). The fungi tried here made moderate growth on this amino acid excepting *I. toruloides* which grew well on it.

Peptone, in the concentration used, was found to be a good source for *A. aplanes* and *I toruloides* and only moderate for *I. unispora* and *S. parasitica.* A part of its virtue may be ascribed to its complex nature, for a mixture of nitrogen sources may be better utilized than a single source. Peptone also contains most of the water soluble vitamins (Stokes *et al* 1944).

SUMMARY

1. The utilization of different nitrogen sources by *Achlya aplanes, Isoachlya unispora, I. toruloides* and *Saprolegnia parasitica* was studied under controlled conditions.

2. These organisms were unable to utilize nitrite or nitrate nitrogen but grew best on ammonium nitrate. Ammonium sulphate was poorly utilized by all fungi except *I. unispora* for which it was a moderate source.

3. The behaviour of the present fungi towards the choice of different organic nitrogen compounds, varied diversely. Of the five monoamino monocarboxylic acids, serine proved best for *Achlya aplanes,* followed by alanine, glycine, valine and leucine. Alanine proved the most favourable source for *I. unispora* followed by valine, glycine, leucine and serine. Valine was best for *I. toruloides* followed by leucine, alanine, serine and glycine. Leucine was best for *S. parasitica* followed by valine, glycine, alanine and serine. Of the two monoamino dicarboxylic acids glutamic acid served as the most favourable source for the growth of all the organisms. Asparagine was utilized by all the fungi in varying degrees. Both arginine and histidine proved to

be good sources of nitrogen while cystin and acetamide were utilized fairly. Pepton in the concentration used was found to be a favourable source of nitrogen.

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A NEW SPECIES OF GALL MIDGE (ITONIDIDAE : DIPTERA) FROM INDIA

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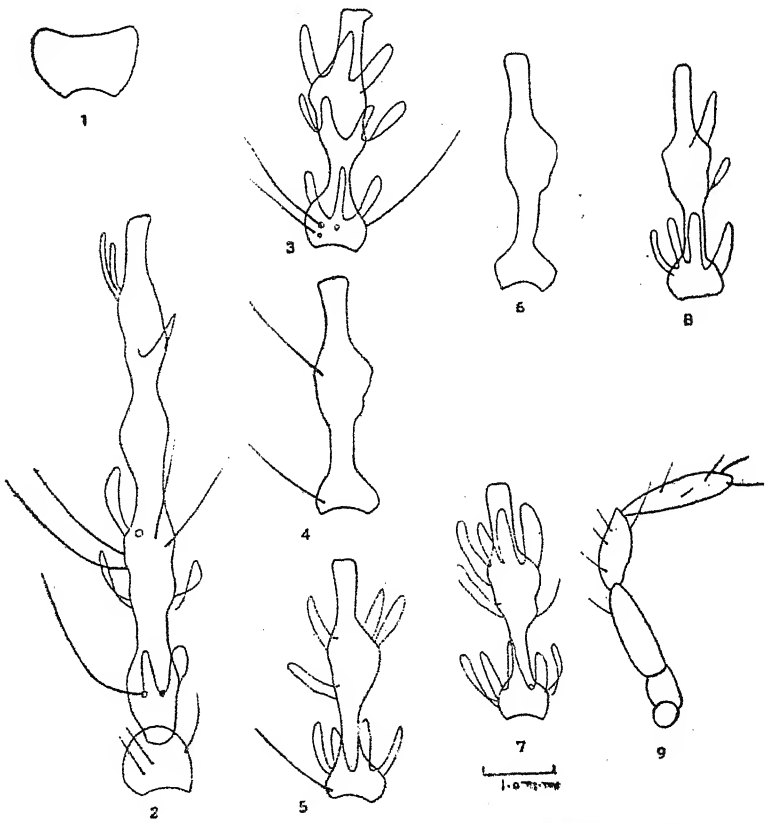
SUBFAMILY ITONIDIDINAE
TRIBE TRIFILINI

Charidiplosis Tavares was erected in 1918 with *C. coninna* Tavares as the type. Rao (1953, 1956) recorded this genus for the first time from India and described. two species. I describe here a third species.

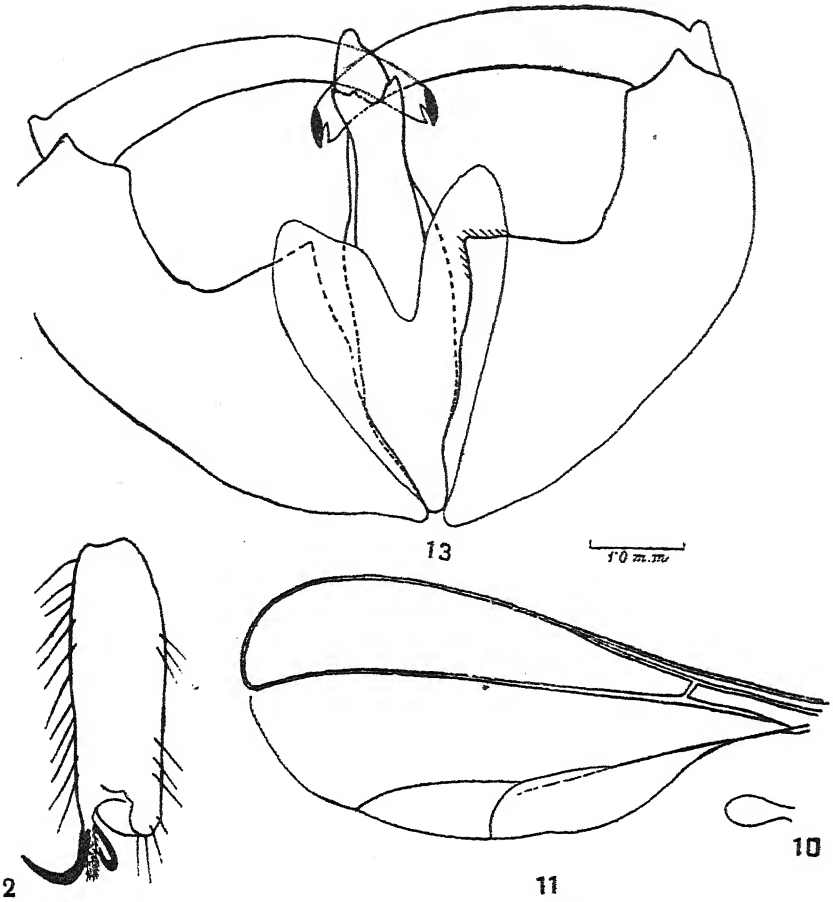
Charidiplosis santhali, sp. nov.

Male.—Length 1·2 mm. Light brown ; eyes confluent above. Trophi slightly produced : palpus (Fig. 9) quardriarticulate, greyish-brown, very sparsely setose, long ; first segment cylindrical, short, length one and three-fifths of its maximum thickness ; second segment cylindrical, longer than the first, length a little less than thrice the maximum thickness ; third segment a little shorter than the second, cylindrical, length two and three-fifths of its maximum thickness ; fourth segment sub-cylindrical, thicker sub-apically than at the base, length a little less than five times its maximum thickness at middle. Antenna light brown, segments graudally becoming thinner and shorter apically, trinodose ; basal enlargements nearly globose, with one whorl of regular circumfila and one whorl of long setae, apical enlargements wider apically and slightly constricted in the middle, with two whorls of regular circumfila and one whorl of setae ; first segment (Fig. 1) pale-brown, wider apically, nearly rectangular, length three-fourths the maximum thickness at apex ; second segment (Fig. 2) shorter than the first, nearly globose ; third segment (Fig. 2) confluent with the fourth, with a small stem at the base, segment longer than the first and second segments combind; basal enlargement sub-globose, one-fourth the length of segment and nearly one and one-half times its maximum thickness, basal stem shorter than the basal enlargement, a little more than half the length of enlargement and one and one-half times its maximum thickness at middle, apical enlargement longer than basal, a little less than twice the maximum thickness, apical stem longer than basal stem and a little more than half the length of apical enlargement and twice as long as thick at the middle : fourth segment (Fig. 2) slightly shorter than the third, basal enlargement globose, one-fourth the length of segment and a little longer than broad, basal stem similar to the basal stem of the preceding segment, apical enlargement as long as that of the third segment but slightly broader, length one and two-thirds of its maximum thickness, apical stem longer than that of the third segment, nearly twice as long as broad ; fifth segment (Fig. 3) slightly shorter than the fourth, basal enlargement a little less than one-fifths of the segment and wider than long, sub-globose, basal stem with length a little less than twice its maximum thickness at middle, apical enlargement nearly one and one-half times the length of basal enlargement and also of its own thickness, apical stem a little less than apical enlargement

and two and a half times as long as thick; seventh segment (Fig. 4) a little shorter than the fifth, basal enlargement wider than long, length a little more than one-fourth the length of segment, basal stem as that of the fifth segment, apical enlargement one and one-thirds as long as thick, apical stem a little shorter than the apical enlargement and longer than the basal stem, length three and one-thirds of its own thickness; eighth segment (Fig. 5) as long as the fifth segment but slightly narrower than the latter; ninth segment (Fig. 6) as long as seventh segment, basal enlargement and basal stem narrower, apical enlargement and apical stem similar to that of the seventh segment; tenth segment (Fig. 7) shorter than the ninth, basal stem one-fifths of the length of segment, wider than long apical enlargement narrower and shorter than that of the ninth segment; twelfth-segment (Fig. 8) as long as tenth segment, apical enlargement one and one-fourths of its maximum thickness, apical stem as long as apical enlargement, length five times its maximum thickness. Mesonotum brown; scutellum and post scutellum pale-brown; halteres (Fig. 10) light brown. Wing (Fig. 11) hyaline,



1. Scape.
2. Pedicel, third and fourth antennal segments.
3. Fifth antennal segment.
4. Seventh antennal segments.
5. Eighth antennal segment.
6. Ninth antennal segment.
7. Tenth antennal segment.
8. Twelfth antennal segment.
9. Palpus.



10. Halter.
11. Wing.
12. Hind claw.
13. Male genitalia.

neither too long nor too broad, length one and a-half times its maximum breadth, costa interrupted at its union with vein R_5 and the latter slightly curved and reaching the wing margin well beyond the apex, vein R_1 uniting with costa before the middle of wing, R_5 present, vein M_{1+2} forked. Legs long, palish-brown, moderately hairy, Metatarsus slightly shorter than the terminal tarsal segment, second tersal segment longer than the following segments combined ; claw (Fig. 12) dark brown, simple, slender, bent at right angles ; empodium slightly shorter than the claw. Genitalia (Fig. 13) palish brown, basal clasp segment with an inner triangular basal lobe, nearly two and one-fourths times as long as broad, terminal clasp segment shorter and slender than the basal clasp segment, wider at base, evenly curved and tapering towards the apex and ending in a blunt tooth ; dorsal plate shorter than the ventral plate, broadly and deeply incised in the middle, lobes beset with small setae, ventral plate narrower than the dorsal plate, a little less than one and a half times the length of dorsal plate, broadly and shallowly incised in the middle, apex with four pointed tips, length nearly six times the maximum thickness, style longer than the ventral plate and shorter than the basal and terminal clasp segments.

Holotype : One male dissected and mounted on slide in the collection of the author and labelled, "at light," coll P. Grover, July 1960, Mihijam, Santhal pargana, Bihar.

This species differs from the two known species in the following : proportion of palpal and antennal segments, distal antennal segments trinodoes, vein R_5 distinct, vein R_5 slightly curved, metatarsus shorter than the terminal tarsal segment, second tarsal segment longer than the following segments combined, empodium a little shorter than the half the length of claw, ventral plate shallowly and broadly incised in the middle with four apically pointed tips, style longer than ventral plate and shorter than basal clasp segment.

ACKNOWLEDGMENT

This work was planned and carried out in the Zoological laboratories of the University of Allahabad under the guidance and supervision of Dr. S. N. Prasad to whom the present worker expresses her gratefulness. She is indebted to Prof. M. D. L. Srivastava, Head of the Zoology Department for facilities. The author wishes to express her gratitude to Dr. S. N. Rao for encouragment and help on various occasions. Her thanks are also due to Government of India for the award of a Junior research scholarship.

KEY TO SPECIES

1. First three palpal segments similar to one another; fourth antennal segment as long as third ; ventral plate shallowly incissed, style longer than ventral plate, empodium shorter than claw .. *indica* Rao
   first three palpal segments unequal ; fourth antennal segment shorter than third ; empodium half the length of claw or shorter than claw ; style longer or shorter than the basal clasp segment .. 2.
2. Vein R_5 faint, vein R_5 straight ; metatarsus equal to terminal tarsal segment ; empodium half the length of claw, style longer than the basal clasp segment, ventral plate deeply incised .. *orientalis* Rao
   Vein R_5 distinct, vein R_5 curved ; metatarsus shorter than the terminal tarsal segment ; empodium slightly shorter than half the length of claw ; ventral plate shallowly and broadly incised, style shorter than basal clasp segment .. *santhali*, sp. nov.

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TOXIN PRODUCTION BY *FUSARIUM ORTHOCERAS* VAR. *CICERI* CAUSING WILT OF GRAM

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INTRODUCTION

Views expressed in literature on the mechanism of vascular wilts caused by pathogenic *Fusaria* centre round the controversy whether it is primarily due to a mechanical blocking of the vascular track by mycelial growth and gummy substances, formed by the host parasite interaction, or due to the effect of specific toxins produced by the parasite. Young and Bennett (1921) have pointed out that wilting of potato plants may be produced by a water solution of an alcoholic precipitate derived from 28 days' culture of *Fusarium oxysporium* in Richard's medium. Fahmy (1923) observed that *Fusarium solani* gives out a toxin having the same property against plants. Rosen (1926) suggested that an inorganic salt in the form of nitrite is one of the toxic ingredients inducing wilt of cotton caused by *Fusarium vasinfectum*. That *Fusarium lycopersici* causing wilt of tomato can produce toxic substance was reported by Haymaker (1928). Working on wilt of pea caused by *Fusarium orthoceras* var. *pisi* Linford (1931) has shown that filtrates from cultures of the fungus in Richard's solution produce a type of rapid necroses in cut shoot of pea. In recent years toxins have figured prominently in the literature of wilt diseases (Gottlieb 1944, Diamond and Waggoner 1953*a*, Gaumann 1951, 1957, Sadasivan and Subramanian 1954). Gaumann (1951) attributed the wilt symptoms in tomato to an irreversible destruction of the osmotic prerequisite for turgor by the action of a systemic toxin-lycomarasmin produced by *Fusarium lycopersici* Sacc. Following the earlier findings of Scheffer and Walker (1953), Gothoskar *et al* (1953 and 1955) reported that extracts of young bran cultures of *Fusarium lycopersici* rich in pectin methyl esterase could reproduce typical wilt syndorne in tomato shoot cuttings and these findings were further amplified by Winstead and Walker (1954).

Many prominent workers on the problem (Diamond and Waggoner 1953*b*, Lakshminaryanam and Subramanian 1955, Kalyansundaram and Venkta Ram 1956, Kern and Kluepfel 1956) have shown that *Fusarium* culture filtrates in general produce ethylene and Fusaric acid, the only two compounds discovered upto this day having vivotaxin property.

The culture of *Fusarium orthoceras*, a soyabean wilt causing organism, has been noted to produce a non-volatile substance which is toxic to soyabean plants (Neely 1957). Quite recently, as indicated by Sadasivan (1958), Venkta Ram (1957) has demonstrated and produced evidence to believe that under optimum conditions *Fusarium orthoceras* synthesised Fusaric acid in sufficient quantity. It has also been shown that the quantity of the Fusaric acid formed is more if the reaction of the medium is either neutral or alkaline. Further, it has been pointed out by him that 5 to 10% diluted filtrate of *Fusarium orthoceras* produced the typical vein clearling—an early symptom of wilts.

In the present work experiments have been done to see if any toxin is formed in the culture filtrate of *Fusarium orthoceras* var. *ciceri* causing wilt of gram. Further, the production of toxin in relation to age of culture filtrate and temperature of incubation was investigated. Also the toxicity of the filtrate was studied in relation to boiling and steam sterilization. The effect to adding some antibiotics in the basal medium on production of toxin was observed.

METHOD AND MATERIAL

Fusarium orthoceras var. *ciceri* obtained from wilted plants of gram was inoculated in Richard's liquid medium and incubated at 27° to 29°C. After three weeks' growth the fungus mat was filtered out and the filtrate was tested for the production of toxin. Gram seedlings previously growing in normal Shive Culture solution were transferred to another set of tubes containing various concentrations of the filtrates. The roots were provided with proper condition of darkness by covering the tubes by black paper. In control series the seedlings were transferred in water. It was also determined that there is no chemical constituent in Richard's medium which is toxic to the growth of gram plants in the given concentrations. Wilting of different degrees was outlined as below in increasing order :—

1. Turgid	.. indicated as	..	T
2. Sagging	.. „	..	S
3. Partial Drooping	.. „	..	Dp
4. Drooping	.. „	..	D
5. Yellowing of leave	.. „	..	Y
6. Wilting	.. „	..	W

For seeing the influence of age of culture on toxin production the fungus was grown in Richard's liquid medium for 12, 18, 24, and 30 days and the respective filtrates were tested for their toxicity. The filtrate obtained from twenty one days old culture was boiled for five minutes and sterilized for ten minutes at 6 lbs. steam pressure to see their effect on the toxicity of the filtrates. The retention of toxicity on dilution was studied by diluting the filtrate with sterilized bidistilled water in proportions of 6 : 0, 5 : 1, 4 : 2, 3 : 3, 2 : 4 and 1 : 5. The effect of varying temperatures on the toxin production was studied by incubating the cultures growing in Richard's liquid medium at 17°, 20°, 25°, 28°, and 31°C (± 1°C) respectively, for three weeks. 25 c.c. of each of the antibiotics (Ambramycin 'A', Chloromycetin 'B', Penicillin 'C', Streptomycin 'D' and Terramycin 'E') in double the required concentrations were mixed with 25 c.c. of 2N Richard's medium so as to obtain four concentrations *viz.* 25 p.p.m., 50 p.p.m., 75 p.p.m. and 100 p.p.m. before inoculating the fungus and the filtrate of twenty one days' old culture was tested for production of toxin.

RESULTS

Observations recorded for toxin production in 21 days' old filtrate revealed that gram seedlings showed various degrees of wilting and finally they died, while in the control which was kept in water they were turgid indicating that the toxins are produced by *Fusarium orthoceras* var. *ciceri* in Richard's liquid medium. Further, the effect of various factors on the toxin production is recorded below.

TABLE I

Effect of period of growth of the fungus on toxin production

Period of growth	Symptoms on seedlings in hours									
	4	8	12	18	24	36	48	60	72	84
12 Days	T	T	T	T	T	T	S	D	Y	W
18 Days	T	T	S	Dp	D	D	Y	W	..	..
24 Days	T	S	D	Y	W	..	..	..	..	..
30 Days	S	D	Y	W	..	..	..	..	..	..
Water, Control	T	T	T	T	T	T	T	T	T	T

It will be seen from table I that in 12 days' old culture filtrate the seedlings were still turgid after 36 hours and complete wilting occurred only after 84 hours. Eighteen days' old filtrate, however, was more toxic to the plants and their leaves began sagging after 12 hours and complete wilting was noticed in 60 hours. The filtrate of 24 days' old culture was still more toxic because sagging was observed only after 8 hours, while complete wilting set in within 24 hours. In thirty days' old filtrate sagging was noticed in 4 hours while it took 18 hours only for complete wilting. The experiments show that the production of the toxin is directly related with the growth of the fungus in the culture medium. In control all the seedlings remained turgid.

TABLE II

Effect of boiling and sterilization of 21 days' old culture filtrate.

Different treatments of filtrates	Symptoms on seedlings in hours				
	10	15	24	36	50
Un-boiled	S	D	Y	W	..
Boiled	S	D	Y	W	..
Sterilized	T	S	D	Y	W
Water, Control	T	T	T	T	T

Both boiled and unboiled filtrates produced rapid drooping of leaves followed by yellowing and ultimately wilting in 36 hours. In both the cases wilting was in acropetal manner, a characteristic symptom of *Fusaria* wilts. The toxic material in the filtrate was not destroyed by boiling showing that it is thermostable. In sterilized filtrate the toxic effect is reduced to some extent because the plants take 50 hours for wilting while in the unboiled series they wilted in 36 hours. All the plants remained turgid in the control.

TABLE III

Effect of dilution of culture filtrate on wilting

Filtrate diluted with water	Symptoms on seedlings in hours													
	10	15	24	36	48	60	72	84	96	108	120	132	144	150
Undiluted	S	D	Y	W	..	..	..	..	..	..	..	..	..	..
5 in 1	S	S	D	Y	W	..	..	..	..	..	..	..	..	..
4 in 2	T	S	S	Dp	D	Y	W	..	..	..	..	..	..	..
3 in 3	T	T	S	S	Dp	D	D	Y	Y	W	..	..	..	..
2 in 4	T	T	T	T	S	S	Dp	Dp	D	Y	Y	W	..	..
1 in 5	T	T	T	T	T	T	T	S	S	D	D	Y	Y	W
Water, Control	T	T	T	T	T	T	T	T	T	T	T	T	T	T

Seedlings placed in undiluted filtrate showed sagging after 10 hours. When five parts of the filtrate were diluted with one part of sterilized bidistilled water plants though started sagging as early as in the undiluted filtrate but did not wilt until after 48 hours. In four parts of the filtrate and two parts of water, sagging was delayed and so also wilting by 24 hours. When the filtrate was diluted with an equal amount of sterilized distilled water sagging was still further delayed and plants did not wilt until after 108 hours. A further dilution of the filtrate with double the quantity of water takes still more time *i.e.* 132 hours for wilting. When it was diluted five times wilting occurred after a period of 150 hours and sagging was also correspondingly delayed. In control series the plants growing only in distilled water remained turgid even after 150 hours but showed signs of loss in vigor. The experiment indicates that the toxin(s) are soluble in water and their effect on the time taken for symptom production is directly related with dilution, the more diluted the solution the longer it takes to produce the characterisitic symptoms of wilt.

TABLE IV

Effect of temperature on the production of toxins in 21 days' old culture of the pathogen

Filtrate from culture grown at different temperatures	Symptoms on seedlings in hours									
	5	10	15	24	36	48	60	72	84	96
31°C	S	D	Y	W	..	..	..	..	..	..
28°C	T	S	D	Y	W	..	..	..	..	..
25°C	T	S	Dp	D	Y	W	..	..	..	..
20°C	T	T	S	Dp	D	Y	Y	W	..	..
17°C	T	T	T	T	S	S	D	D	Y	W
Water, Control	T	T	T	T	T	T	T	T	T	T

From the table IV it is clear that within the range of temperatures studied toxin production is increased at higher temperatures.

TABLE V

Effect of certain antibiotics in different concentrations on the production of toxin in the three week old cultures

Various kinds of filtrates	Symptoms on seedlings in hours											
	10	15	24	36	48	60	72	84	96	108	120	132
Normal	S	D	Y	W	..	..	..	..	..	..	..	..
A 25 p.p.m.	T	T	T	T	T	T	S	S	Dp	D	Y	W
A 50 p.p.m.	T	T	T	S	Dp	D	Y	W	..	..	..	..
A 75 p.p.m.	T	S	D	Y	W	..	..	..	..	..	..	..
A 100 p.p.m.	S	D	Y	W	..	..	..	..	..	..	..	..
B 25 p.p.m.	T	T	S	S	D	Y	Y	W	..	..	..	..
B 50 p.p.m.	T	T	T	T	T	T	T	S	S	D	Y	W
B 75 p.p.m.	T	T	T	S	Dp	D	Y	Y	W	..	..	..
B 100 p.p.m.	T	S	Dp	D	Y	W	..	..	..	..	..	..
C 25 p.p.m.	T	S	Dp	D	Y	W	..	..	..	..	..	..
C 50 p.p.m.	T	S	D	Y	W	..	..	..	..	..	..	..
C 75 p.p.m.	T	S	Dp	D	Y	W	..	..	..	..	..	..
C 100 p.p.m.	T	S	D	Y	W	..	..	..	..	..	..	..
D 25 p.p.m.	S	D	Y	W	..	..	..	..	..	..	..	..
D 50 p.p.m.	S	D	Y	W	..	..	..	..	..	..	..	..
D 75 p.p.m.	S	D	Y	W	..	..	..	..	..	..	..	..
D 100 p.p.m.	S	D	Y	W	..	..	..	..	..	..	..	..
E 25 p.p.m.	S	D	Y	W	..	..	..	..	..	..	..	..
E 50 p.p.m.	S	D	Y	W	..	..	..	..	..	..	..	..
E 75 p.p.m.	T	S	S	D	Y	W	..	..	..	..	..	..
E 100 p.p.m.	T	S	Dp	D	Y	W	..	..	.	..	..	..
Water, Control	T	T	T	T	T	T	T	T	T	T	T	T

A = Ambramycin, B = Chloromycetin, C = Penicillin, D = Streptomycin, E = Terramycin.

The effect of ambramycin in 100 p.p.m., streptomycin in all the four concentrations and terramycin in 25 p.p.m. is the same as that in the normal untreated one. In all these cases the wilting of the plants is noticeable in 36 hours. The toxicity under treatments of ambramycin 75 p.p.m. and penicillin in 50 p.p.m. and

100 p.p.m. is slightly less as complete wilting is observed only after 48 hours. The toxicity of the filtrates is still further reduced in the treatments with chloromycetin (100 p.p.m.) and penicillin (25 and 75 p.p.m.) the wilting occuring after 60 hours. In treatments with ambramycin 50 p.p.m. and chloromycetin 25 p.p.m. the wilting is observed when the seedlings were kept in the filtrates for a period as long as 84 hours. 96 hours were taken with the treatment of chloromycetin 75 p.p.m. However the toxic effect was minimum when the treatments of ambramycin 25 p.p.m. and chloromycetin 50 p.p.m. were employed. All the seedlings remained perfectly turgid in the control that is when kept in water alone. These results indicate that the production of toxin and its toxicity is altered under the influence of some antibiotics although a few antibiotics had no effect at all.

DISCUSSION

Damage to absorbing organs has been suggested by Orton (1902) as a principal factor in wilts. But this does not seem to be general in all the wilt diseases. In most cases the extent of root damage is too meagre to account for such an acute water shortage and, often, wilting occurs long before any visible damage to the root system is noticeable. Also injury to roots aparently does not reduce absorption of water from the soil (Subramanian 1958). Presence of wefts of hyphae, masses of organism, gums and tyloses, gas pockets, the disintergation of vessels walls into the products which increase the viscosity of the tracheal fluid (Diamond 1955) have only a limited influence in bringing about wilting. After the work of Rosen (1926) the production of toxins by wilt causing *Fusaria* was also shown by Kulkarni and Mundkur (1931), Gaumann (1951) and Kalyansundaram (1953). Linford (1931) working on *Fusarium orthoceras* var. *pisi* causing wilt of pea and Venkta Ram (1957) in an unknown strain of *Fusarium orthoceras* demonstrated the production of toxins. That the toxins are produced as well by *Fusarium orthoceras* var. *ciceri* has been observed in the present investigations.

The toxins retain their effect even when the solution containing the toxic substance (s) is boiled, but under steam pressure its toxicity slightly falls. This is in line with the observations of Naim (1958) on cotton wilt. The toxin produced by the pathogen under investigation well stands dilution in bidistilled sterilized water. The toxicity being directly proportional to the concentration of the filtrate, a highly diluted solution taking much longer time to produce wilting than a more concentrated solution, as also observed by Venkta Ram (1957) on *Fusarium orthoceras* and Naim (1958) on *Fusarium* causing wilt of cotton. The production of the toxin in the culture medium by the fungus is affected by temperature, the higher ranges upto 31°C being more favourable than the lower range of 17°C the wilting taking more time in the latter than the former. Similar trends have also been observed by Kalyansundaram and Subba Rao (1957) on the production of vivo-toxin in *Fusarium vasinfectum* causing wilt of cotton.

Out of the five antibiotics used in growing cultures of the pathogen only ambramycin and chloromycetin appear to lower the production of toxin to some extent, while the remaining three—penicillin, and sreptomycin and terramycin are little effective, as also observed by Pramer (1956).

SUMMARY

Experiments conducted in the present investigation indicate that the toxin is produced in the culture filtrate of *Fusarium orthoceras* var. *ciceri* causing wilt of gram. The production of toxin is affected by the period of the growth of fungus in

Richard's culture medium as was demonstrated by growing it for different periods *viz.* 12, 18, 24 and 30 days. It was maximum in 30 days' old culture. The temperature effect was studied by incubating the culture flasks at temperature of 17°, 20°, 25°. 28° and 31°C, the maximum toxic effect having been obtained at 28° and 31°C. Both boiled and unboiled filtrates produce rapid wilting and ultimate death. The toxic substance (s) of the filtrate was sterilized for ten minutes at 6 lbs. pressure it proves slightly less toxic to the plants. The toxin can be diluted and the toxicity being directly proportional to the concentration of filtrate. Five antibiotics (ambramycin, chloromycetin, penicillin, streptomycin and terramycin) in various concentrations were employed for testing their effect on toxin production. When added to the culture medium only ambramycin 25 p.p.m. and chloromycetin 50 p.p.m. were effective in delaying wilt upto 132 hours, while the normal untreated set it was 36 hours only.

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A LIST OF FISHES FROM JABALPUR, M. P., INDIA

By

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The systematic study of the fish fauna of Jabalpur District was started in July, 1954, and continued through the following years. The collections made from all over the district resulted in bringing the number of species to fifty four which may be regarded as being fairly representative of fish fauna of this region.

PHYSICAL FEATURES

Jabalpur District is situated centrally in the State of Madhya Pradesh lying between 22° 49' and 24° 8' N. and 79° 21' and 80° 58' E. It is bordered by Panna and Satna Districts of Madhya Pradesh in the north, Shahdol and Mandla in the east, Seoni in the south and by the district of Damoh in the west. The total area of the district is 3,912 square miles. The terrain is hilly with two principal mountain ranges, the Vindhya Range running in the west and north east and being situated north of the river Nerbudda ; and the Satpura Range situated to the south of the above river.

There are two main river systems in the District :

(1) The Nerbudda system.

(2) The Chhoti Mahanadi system.

The Nerbudda system consists of the main river, the Nerbudda which originates from Amarkantak in the Satpura Range and flows northwards and then westwards in the district before entering Bombay State. The main tributaries of the Nerbudda in Jabalpur District are the rivers Gaur and Hiran.

The Chhoti Mahanadi system consists of Chhoti Mahanadi as the main river arising from the Satpura Ranges in the Mandla District. It enters this district from the south-eastern corner and then persuing a northerly course flows into Rewa, finally to join the Son, a tributary of the Ganges. The rivers Niwar and Katni are its main tributaries flowing through the district.

Most of these rivers take their origin from the hilly tracts and for a greater part may be described as hill streams. The rivers get swollen during the monsoons but for the greater part of the year have comparatively little water.

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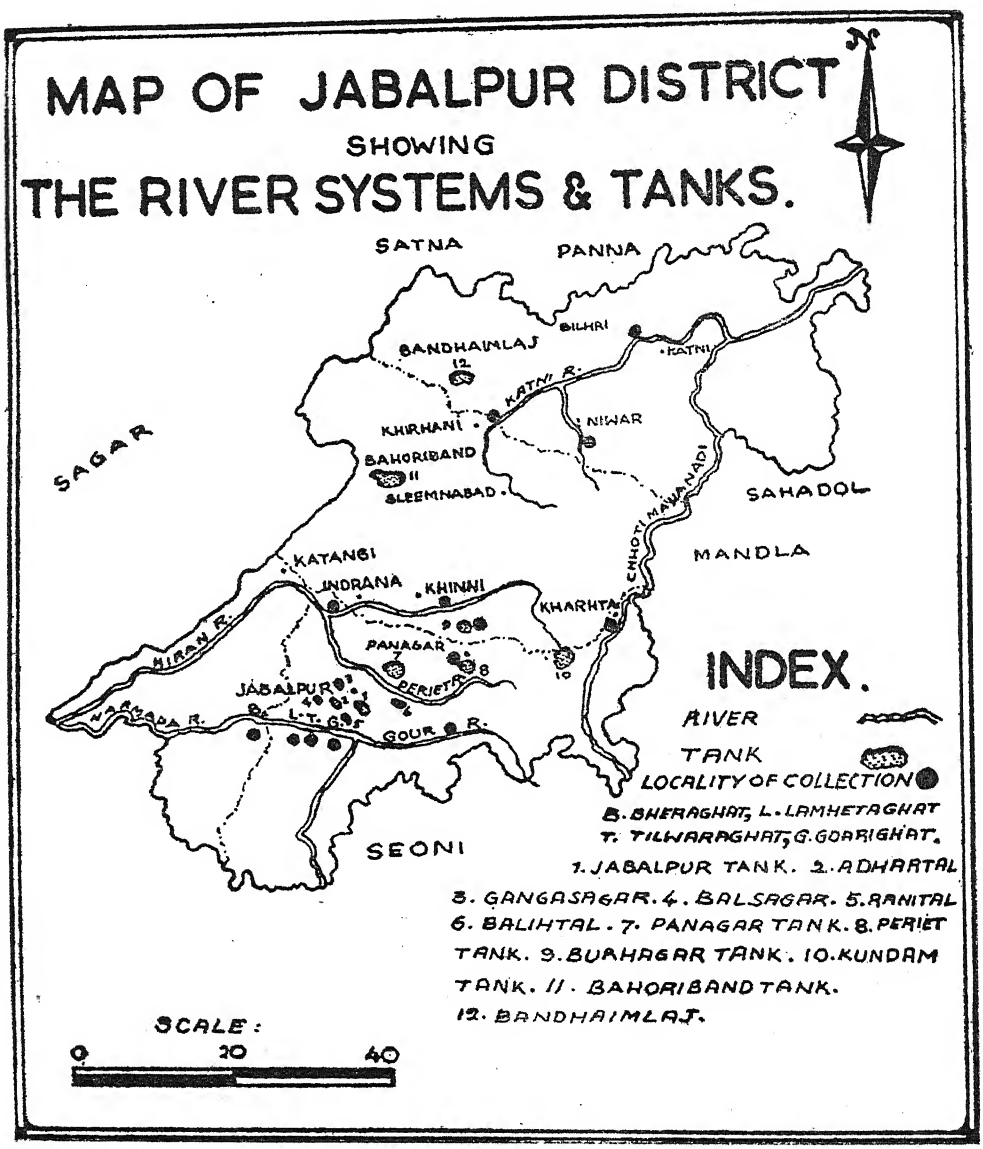
Apart from these two river systems there are a number of lakes and tanks which are perennial. The important ones are the Gokalpur lake, the Adhartal, Ganga-sagar, Balsagar, Ranital, Balehatal Budhagar, Tank, Pariat Tank and Bahoriband Tank. The source of water in these tanks is from the annual rains which is between 55—60 inches per annum for the district. Out of the above tanks Adhartal, Balsagar, Ganga-sagar, etc., are used by the State Fisheries Department for the culture of *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*.



LIST OF SPECIES

A classified list of the fishes along with their Hindi local names is presented below. The scheme of classification followed is according to the one suggested by L. S. Berg (1940, Russian, 1947 English).

Scientific Name		*Local Name*
Order CYPRINIFORMES		
Suborder CYPRINOIDEI		
Family *CYPRINIDAE*		
Sub-family *CYPRININAE*		
1. *Labeo rohita*	(Hamilton)	Rohu
2. *Labeo calbasu*	(Hamilton)	Kalbasu
3. *Schismatorhynchus* (*Nukta*) *nukta*	(Sykes)	Nukta
4. *Labeo boggut*	(Sykes)	Gohria
5. *Labeo gonius*	(Hamilton)	Kurchia
6. *Labeo bata*	(Hamilton)	Bata
7. *Labeo dero*	(Hamilton)	Chilwa
8. *Cirrhinus reba*	(Hamilton)	Rekhara
9. *Cirrhinus mrigala*	(Hamilton)	Mrigal
10. *Cirrhinus cirrhosa*	(Bloch)	Narain
11. *Catla catla*	(Hamilton)	Catla
12. *Puntius amphibius*	(Cuv. and Val.)	Chaptara
13. *Puntius sarana*	(Hamilton)	Potiah, Sinnia
14. (*Barbus tor*) *Tor tor*	(Hamilton)	Mahseer
15. *Puntius ticto*	(Hamilton)	Potiah
16. *Puntius stigma*	(Hamilton)	Potiah, Karwari
17. *Puntius tetrarupagus*	(McClell)	Durhi
18. *Puntius sophore*	(Hamilton)	Potiah
19. *Garra lamta*	(Hamilton)	Patharchata
20. *Rohtee cotia*	(Hamilton)	Koti; Muchnee
21. *Barilias bendelisis*	(Hamilton)	Johra
22. *Aspidoparia morar*	(Hamilton)	Chilwa, Gulthi.
23. *Rasbora daniconius*	(Hamilton)	Khouli, Dundi
24. *Oxygaster bacaila*	(Hamilton)	Chelliah
25. *Oxygaster clupeoides*	(Bloch)	Roopchal, Alkut
Family *COBITIDAE*		
Sub-family *NEMACHILINI*		
26. *Noemacheilus botia*	(Hamilton)	Teli-murra

Scientific Name		*Local Name*
Sub-family *COBITINI*		
27. *Lepidocephalichthys guntea*	(Hamilton)	Gur-gutchi
Suborder *SILUROIDEI*		
Family *SILURIDAE*		
28. *Wallagonia attu*	(Bloch and Schneider)	Padan, Boyari
29. *Ompok bimaculatus*	(Bloch)	Pufta, Gugli
Family *BAGRIDAE*		
30. *Mystus seenghala*	(Sykes)	Singhara
31. *Mystus cavasius*	(Hamilton)	Katurna
32. *Mystus vittatus*	(Bloch)	Tengra
33. *Rita rita*	(Hamilton)	Rita
34. *Rita Pavimentata*	(Val.)	Ghagra
Family *SCHILBEIDAE*		
35. *Eutropiichthys vacha*	(Hamilton)	Batchua
Family *SACCOBRANCHIDAE (HETEROPNEUSTIDAE)*		
36. *Heteropneustes fossilis*	(Bloch)	Sigan
Family *CLARIIDAE*		
37. *Clarias batrachus*	(Linn.)	Magura
Family *SISORIDAE*		
38. *Bagarius bagarius*	(Sykes)	Goonch
39. *Glyptothorax telchitta*	(Hamilton)	Gangai
Order *OPHIOCEPHALIFORMES*		
Family *OPHIOCEPHALIDAE (CHANNIDAE)*		
40. *Channa (Ophicephalus) punctatus*	(Bloch)	Karra, Gurrai
41. *Channa (Ophicephalus) gachua*	(Hamilton)	Dhok
42. *Channa (Ophicephalus) striatus*	(Bloch)	Maral, Kabra
43. *Channa marulius*	(Hamilton)	Phul maral
Order PERCIFORMES		
Suborder GOBIOIDEI		
Family *GOBIIDAE*		
44. *Glassogobius giuris*	(Hamilton)	Kharpa, Bulla

Scientific Name		*Local Name*
Suborder PERCOIDEI		
Family *CENTROPOMIDAE*		
45. *Ambassis ranga*	. . (Hamilton)	. . Janjhra, Kanghi-machhli.
Family *NANDIDAE*		
46. *Badis badis*	. . (Hamilton)	. . Kali Potiah
47. *Nandus marmoratus*	. . (Cuv. and Val.)	. . Chamaria, Cha-mar-machhli.
Order MUGILIFORMES		
Suborder MUGILOIDEI		
Family *MUGILIDAE*		
48. (*Liza*) *Rhinomugil corsula*	. . (Hamilton)	. . Arwari
Order MASTACEMBELIFORMES		
Family *MASTACEMBELIDAE*		
49. *Mastocembalus armatus*	. . (Lacépède)	. . Balm
50. *Mastocembalus pancalus*	. . (Hamilton)	. . Gurchee
51. *Macrognathus* (*Rhyncobdella*) *aculeatum* (Bloch)		. . Gaichee
Order CLUPEIFORMES		
Suborder NOTOPTEROIDEI		
Family *NOTOPTERIDAE*		
52. *Notopterus notopterus*	. . (Pallas)	. . Pulli, Chambaree
53. *Notopterus chitala*	. . (Hamilton)	. . Chilul
Order BELONIFORMES		
Suborder SCOMBERESOCOIDEI		
Family *BELONIDAE* (*XENENTODONTIDAE*)		
54. *Xenentodon cancilla*	. . (Hamilton)	. . Sooja

FISHES OF ECONOMIC VALUE

Most of the species being of small size are not of much economic importance. Only ten species can be considered of economic value as food fishes and they all belong to the carnivorous type. These ten species arranged in numerical sequence of their importance are as follows :

1. *Mastocembalus armatus*
2. *Channa* (*Ophicephalus*) *punctatus*
3. *Channa* (*Ophicephalus*) *Striatus*
4. *Channa* (*Ophicephalus*) *marulius*
5. *Mystus seenghala*
6. *Mystus vittatus*
7. *Wallagonia attu*
8. *Eutropiichthys vacha*
9. *Notopterus notopterus*
10. *Notopterus chitala*

Of these, the first four are well established and breeding conditions for them in most of the tanks seem to be quite favourable. The fishes which feed on mosquito larvae are also of great economic value if we consider the toll taken by malaria every year. *Puntius ticto, Puntius stigma, Rasbora daniconius, Oxygaster bacaila, Oxygaster-clupeoides, Lepidocephalichthys guntea,* as well as the fry of *Mystus vittatus* and *Ophicephalus punctatus* found in practically all the tanks, feed on mosquito larvae and help in keeping down the number of mosquitoes. These are thus larvicidal fishes.

CONCLUDING REMARKS

The fish fauna of Jabalpur shows paucity of carps of economic value, excepting the mahser (*Barbus tor*) *Tor tor*. The introduction of the larger species of carps such as *Labeo rohita, Labeo calbasu, Catla catla* and *Cirrhinus mrigala* in the tanks is highly desirable from the piscicultural point of view. Few breeding grounds of major carps have been located in any of the two river systems. The State Fisheries Department imports the carp seed from Bengal and Orissa, in which case there is great mortality of the fish seed. Extensive investigations and survey work may bring to light the breeding grounds of carps in this region.

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FUNGI CAUSING PLANT DISEASES AT JABALPUR (MADHYA PRADESH)—VI. SOME *CERCOSPORAE*

By

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In the first five parts of the paper (listed under reference) 119 fungi causing plant diseases at Jabalpur and its suburbs have been reported. These include 13 *Cercosporae*. The present paper describes 8 more *Cercosporae* causing leaf spots at Jabalpur. It consists of two new species, four new *Cercosporae* for India, one new host record and one new fungus record for the state.

The number of the species are the serial numbers of the fungus flora of Jabalpur.

120. *Cercospora elaeodendri* Agarwal and Hasija sp. nov. on leaves of *Elaeodendron glaucum* Pers., Mahakoshal Mahavidyalaya grounds, September, 1959, Leg. G. P. Agarwal.

Symptoms of the disease

The disease first appears as dark brown pin head spots on any part of the leaf. Spots become circular to angular and increase in size up to 2·5 cm. in diameter. At maturity the central region becomes dark brown bounded by a light brown margin. Spots rarely coalesce. Midrib and the chief veins are freely traversed.

The causal organism

Conidiophores light brown, short, erect, simple or branched, nonseptate to septate, in clusters, 12·4—34·1×3·1—3·9 μ, average 22×3·7 μ; conidia olivaceous, filiform, tapering towards apex, 3—11 septate, straight or curved, scars present, 21·7—80·6×3·1—3·9 μ, average 58·6×3·7 μ (Figs. 1 and 2).

The material was examined by Mr. Deighton of the Commonwealth Mycological Institute, Kew, who reported, "No *Cercospora* has, to my knowledge, been described on *Elaeodendron*, and none of the species described on other Celastraceae match this material. I think it is an undescribed species." It is, therefore, being described here as a new species, *Cercospora elaeodendri*.

Cercospora elaeodendri Agarwal and Hasija sp. nov.

Conidiophori pallide brunnei, breves, erecti, simplices vel ramosi, non-septati vel septati, aggregati, 12·4- 34·1×3·1—3·9 μ, medietate 22×3·7 μ; conidia olivacea, filiformia, fastigata ad apicem, 3—11 septata, recta vel curvata, cicatricibus praesentibus, 21·7—80·6×3·1—3·9 μ, medietate 58·6×3·7 μ.

In foliis *Elaeodendri glauci* Pers. ad Jabalpur in India, mense septembri anni 1959, Leg. Agarwal.

The type specimen has been deposited in the Kew Herbarium No. 79006 and in the Botany Department, M. M. V., Jabalpur.

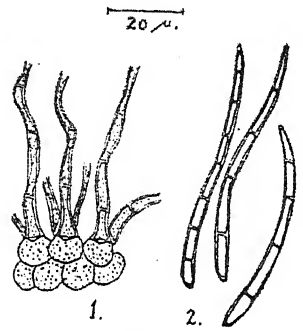
121. *Cercospora agarwalii* Chupp sp. nov. on leaves of *Vitex negundo* L., Berha ghat, Nov., 1959, Leg. Hasija.

Symptoms of the disease

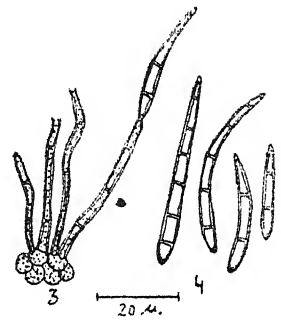
The disease first appears as small dark brown spots on the upper surface of the leaf. It is usually marginal but often starts from apex. The young spots are at first roundish but later on become irregularly angular. As they mature the central region looks dirty brown bounded by a dark brown zone. Some concentric zonations may become evident in the central region. Adjacent spots often coalesce and increase the diseased surface. Midrib and the main veins are freely traversed.

The causal organism

Conidiophores brown, at times paler towards tip, straight or curved, simple or branched, septate, in fascicles, with distinct geniculations, 37·2—130·2×3·1—3·9μ, average 66·7×3·4 μ; conidia hyaline to faintly olivaceous, obclavate, tapering towards apex, 2—7 septate, straight or curved, scars indicating the point of attachment present, 24·8—52·7×3·1—6·2 μ, average 41·7×4·3 μ (Figs. 3 and 4).



Figs. 1—2. *Cercospora elaeodendri, conidiophores and conidia.*



Figs. 3—4. *Cercospora agarwalii, condiophores and conidia.*

The material was examined by Prof. Chupp. He considered it to be a new species, and he deposited the specimen in his Herbarium of the Department of Plant Pathology, Cornell University proposing the specific name as *Cercospora agarwalii* sp. nov. On the authority of Prof. Chupp the present species is described here as *Cercospora agarwalii.*

Cercospora agarwalii Chupp sp. nov.

Conidiophori brunnei, nonnumquam pallidiores ad apices, recti vel curvati, simplices vel ramosi, septati, fasciculati, geniculationibus distinctis ornati, 37·2—130·2×3·1—3·9 μ, medietate 66·7×3·4 μ. Conidia hyalina vel languide olivacea, obclavata, fastigata ad apicem, 2—7—septata, recta vel curvata, cicatricibus monstrantibus punctum unionis praesentibus, 24·8—52·7×3·1—6·2 μ, medietate 41·7×4·3 μ.

In foliis *Viticis negundinis* L. ad Jabalpur in India, mense novembri anni 1959, Leg. Hasija.

122. *Cercospora justiciicola* Tai on leaves of *Justicia simplex*, Waterworks, October, 1959, Leg. Hasija.

Symptoms of the disease

The disease first appears as small decolourised spots on any part of the leaf. The spots become circular to angular and involve nearly half the leaf surface. At maturity the centre of the lesions turns light grey to ash coloured with dark grey margins. Spots often coalesce forming irregular patches. Midrib and the chief veins are freely traversed.

The causal organism

Conidiophores brown, simple, erect, straight or curved, developing in clusters, with geniculations, septate, at times with bulbous base, 31—114·7×3·1—3·9 μ;, conidia hyaline, whip like, tapering towards apex, 3—11 septate, with scars at the basal end, 43·4—170·5×2·3—3·9 μ, average 72·2—3·1 μ.

So far *Cercospora justiciicola* has not been reported from India. It is a new fungus record for the country. *Cercospora acanthacearum* Govindu and Thirum. has been described on leaves of *Justicia betonica* L. from Nandi Hills, Mysore by Govindu and Thirumalachar (1954).

The fungus was identified by Prof. Chupp of Cornell University, U. S. A. and Mr. Deighton of Commonwealth Mycological Institute, Kew. The Material has been deposited in Kew Herbarium No. 79007.

123. *Cercospora paramignyae* Thirum. and Chupp on leaves of *Lagerstroemia parviflora* Roxb., Pariat Tank, Sept., 1959, Leg. Agarwal and Hasija.

Symptoms of the disease

The disease first appears as small light coloured spots, which become brown with a bright yellow halo. Spots are circular to angular in shape and generally only 1—3 spots are present on each leaf. At maturity conidiophores bearing conidia appear as small black dots in the central region. Spots seldom coalesce. Midrib forms a barrier.

The causal organism

Conidiophores light brown, short, septate, simple or branched, in fascicles 46·5—90·3×2·3—3·1 μ, average 77·4×2·9 μ; conidia olive to light coloured, 1—12 septate, filiform, with rounded ends, scars at the basal ends present, 15·5—108·5× 3·1—3·9 μ, average 56 · 3·4 μ.

Lagerstroemia parviflora is a new host record for *Cercospora paramignyae* not so far reported. *C. paramignyae* has been described on leaves of *Paramignya* sp. from Mysore by Thirumalachar and Chupp (1948).

The species was identified by Mr. Deighton. The material has been deposited in Kew Herbarium No 79003.

124. *Cercospora neriella* Sacc. on leaves of *Nerium oleander* L., Pariat Tank and Napier Town, September, 1959, Leg. Hasija.

Symptoms of the disease

The disease first starts as light brown spots from any part of the leaf, but usually from the margins. Spots form irregular dark brown patches on the blade. The central region becomes light grey. More than half the leaf gets involved.

The causal organism

Conidiophores brown, septate, 34·1—179·8×3·1—3·9 μ, average 81·7×3·3 μ, conidia olivaceous, 1—4 septate, filiform, 15·5—65·1×2·3—3·9 μ, average 41·2×3·1 μ.

Cercospora nerii-indici Yamamato has been reported on leaves of *Nerium oleander* from India by Thirumalachar and Chupp (1948) and *C. neriella* Sacc. has been reported on leaves of *Nerium oleander* from Bihar in India by Sydow and McRae (1930). It is a new record for the state.

The species was identified by Mr. Deighton.

125. *Cercospora hyalospora* Muller and Chupp on leaves of *Sida cordifolia* L., Waterworks, October, 1959, Leg. Hasija.

Symptoms of the disease

The disease first appears as pale coloured spots from margin, apex or leaf blade. Spots are irregular, with central region dark brown in which become evident clumps of conidiophores as black dots. Spots rarely coalesce. Main veins are freely traversed. Such spots appear only on the upper surface of the leaf and at its lower surface are lesions of a rust (*Puccinia heterospora* Berk. and Curt.).

The causal organism

Conidiophores brown, simple or branched, erect, straight or curved, with distinct geniculations, septate, 58·9—213·9 × 3·1—3·9 μ, average 123·1 × 3·4 μ; conidia hyaline, tapering towards apex, scars at the basal end, 3—16 septate, 34·1—250×2·3—3·9 μ, average 128·7×3·1 μ.

The organism has been identified as *Cercospora hyalospora* by Prof. Chupp and Mr. Deighton. Mr. Deighton reports, "A similar *Cercospora* is not uncommon in West Africa on the upper surface of *Puccinia heterospora* spots. It is a secondary fungus on the spots, whereas *C. hyalospora* is described as the primary cause of a leaf spot." In the present case also *C. hyalospora* is not a secondary fungus but instead is the primary cause of the leaf infection.

So far there is no record of *Cercospora hyalospora* from India. It is a new fungus record for the country. The material has been deposited in the Kew Herbarium No. 79008*a*.

126. *Cercospora sonchi* Chupp on leaves of *Sonchus arvensis* L., Simla Hills, September, 1959, Leg. Hasija.

Symptoms of the disease

The disease first appears as pin head spots only on the upper surface of the leaf. Spots become dark brown, irregular and may cover half the leaf surface. Chief veins are freely traversed.

The causal organism

Conidiophores brown, short, simple, septate, in clusters, with distinct geniculations, 43·4—93× 3·9—5·4 μ, average 67·6×4·6 μ; conidia very faintly olivaceous, tapering towards the apex, up to 13 septate, scar at the basal end, 37·2—145·7 × 3·1—4·7 μ, average 78·1 × 3·8 μ.

So far there is no record of *Cercospora sonchi* from India. It is a new fungus record for the country.

The species was identified by Prof. Chupp.

127. *Cercospora zinniae* Ell. and Mart. on leaves of *Zinnia panciflora* L., Waterworks, October, 1959, Leg. Hasija.

Symptoms of the disease

The disease first appears as small brown pin head spots from any part of the leaf. Spots become irregular with the central region ash coloured bounded by a dark brown or violet margin and cover almost the whole leaf. At maturity the central region breaks away forming holes. Midrib forms a barrier.

The causal organism

Conidiophores brown, simple or branched, erect, straight or curved, in fascicles, septate, with geniculations, 55·8—156·5 × 3·1—6·2 μ, average 85·5 × 4·3 μ ; conidia hyaline to light coloured, filiform, 2—13 septate, tapering towards apex, scars present. 18·6—204·6 × 1·6—3·1 μ, average 74·5 × 2·8 μ.

So far there is no record of *Cercospora zinniae* from India. It is a new fungus record for the country. The material has been deposited in Kew Herbarium No. 79009.

SUMMARY

The present paper describes 8 *Cercosporae* causing leaf spots at Jabalpur. It includes *Cercospora elaeodendri* Agarwal and Hasija on *Elaeodendron glaucum* Pers., *C. agarwalii* Chupp on *Vitex negundo* L., the two new species. *C. justiciicola* Tai on *Justicia simplex*, *C. hyalospora* Muller and Chupp on *Sida cordifolia* L., *C. sonchi* Chupp on *Sonchus arvensis* L., and *C. zinniae* Ell. and Mart. on *Zinnia panciflora* L., four new fungus records for India. *Lagerstroemia parviflora* Roxb. is reported to be a new host for *C. paramignyae* Thirum. and Chupp. *C. neriella* Sacc. on leaves of *Nerium oleander* L., is a new record for the state.

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NEW SUPERFAMILY ORCHIPEDIOIDEA (SUBORDER ; ECHINOSTOMATA SZIDAT, 1939 ; ORDER ECHINOSTOMIDA LA RUE, 1957).

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[Recieved on 22nd September, 1961]

The family Achillurbainiidae Dollfus, 1939 is dropped. It is synonymous to the family Orchipedidae Skrjabin, 1924. The genus *Achillurbainia* Dollfus, 1939 closely resembles *Orchipedum* Braun, 1901 in the absence of prepharynx, presence of short oesophagus and undulating caeca, pre-equatorial acetabulum slightly larger than oral sucker and numerous testes. In *Orchipedum* the latter are entirely post-ovarian and intracaecal but in *Achillurbainia* they are both extracaecal and intracaecal. Cirrus sac and cirrus are absent and strongly winding vesicula seminalis lies in preacetabular region. Genital pore is median post-bifurcal, ovary is submedian and postacetabular, uterus lies in acetabulovarian zone and eggs are large in both. The vitellaria are composed of numerous small follicles, but they are much more extensive in *Achillurbainia*, where they extend also medianwise interrupted by ovary, shell gland complex, acetabulum and vesicula seminalis. The excretory vesicle is tubular and long. The differences between the genra do not entitle their inclusion in separate families. Orchipedidae contains parasites of birds and mammals and belongs to Orchipedioidea n. supf. Dollfus thinks that *Achillurbainia* shows remote affinity with *Paragonimus* Braun, 1899. Orchipedioidea n. supf. probably stands near Gymnophallid or *Parvatrema* ancestor (Gymnophallidae, Fellodistomatoidea, Fellodistomata) or ancestor of Plagiorchiata as it bacame evolved from some echinostomid ancestor. Dollfus (1935) traced the development of *Distoma isostoma* (Rud.) into *Orchipedum isostoma* in the nasal fossa of cat *Felis maniculata domest*, *Mustella vugaris* and fox *Canis vulpes*.

Diagnosis : Echinostomida ; Fellodistomata or Echinostomata. Distomate, unspinate. Acetabulum pre-equatorial, subequal or larger than oral sucker. Prepharynx absent ; oesophagus very short or absent ; caeca undulating or serpentine teminating near or at end. Genital pore median, postbifurcal or preacetabular. Testes divided into numerous follicles. Cirrus sac and cirrus absent. Vesicula seminalis strongly winding, preacetabular. Ovary postacetabular. Receptaculum seminis present. Vitellaria follicular, profuse, extending in hind body or greater part of body. Uterus winding, preovarian, intercaecal. Eggs large. Excretory vesicle tubular, long. Cercaria unknown. Metacercaria with Y-shaped excretory vesicle parasitic in Crustacea. Parasitic in birds and mammals. Family Orchipedidae Skrjabin, 1924 syn. Achillurbainiidae, Dollfus, 1939.

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A CONTRIBUTION TO THE BIBLIOGRAPHY OF GRAMINEAE

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In 1897 Hooker published the first consolidated account of the Gramineae of India. Prior to this, the work on Indian agrostology was represented by a few publications by Symonds, Coldstream, Duthie, Lisboas and Gamble.

With the publication of several monographs on grasses in different parts of the world, stabilization of rules of nomenclature during the current century and finally the changed Indian political boundaries, the picture of Gramineae of India, since Hooker, has considerably changed. It has become necessary to revise the Indian Gramineae in the light of such works as Lecomte's Flora of Indo-China (Grasses by Camus and Camus), Prain's Flora of Tropical Africa (Grasses by Stapf and Hubbard), Konarov's Flora of U. R. S. S. (Grasses by a number of workers), Pilger in Engler and Prantl's Die Naturlichen Pflanzenfamilien and several papers on Indian grasses by Stapf, Chase, Hitchcock, Camus, Hubbard, Bor, Stewart, Raizada, Janaki Ammal, Jain, Bharadwaja, Majumdar, Tiwari and others.

The grasses of Bihar and Orissa, Madras, Bombay, Assam, North-Western India (Punjab and Kashmir) parts of Madhya Pradesh and Bengal have more recently been described by Haines (1924), Fischer (1934), Blatter and McCann (1935), Bor (1940), Stewart (1945), Tiwari (1954) and Majumdar (1956) respectively. Bor's recent book on Grasses (1960) is a monumental work. With the exception of a few short lists there is no recent complete account of the grasses of Rajasthan Kutch, Bengal, Madhya Pradesh, Hyderabad and Mysore.

The Flora of the Upper Gangetic Plain (Families Ranunculaceae to Juncaceae) by J. F. Duthie appeared in parts during the period 1903 to 1920. The Families Palmae to Aroideae and Alismaceae were completed by Duthie before his death in 1922 ; these and others upto Cyperaceae were published by R. N. Parker and W. B. Turrill in 1929. Since Duthie was amongst the foremost workers on the Grasses of India he would have treated Gramineae in his Flora in an excellent manner. He had made extensive collections of the grasses of north-western India and a large number of them is now in the herbarium of the Forest Research Institute, Dehradun. It appeared from grass specimens and some notes in the Dehradun Herbarium that Parker had started the study of this group. Bor in 1940 published a detailed account of 92 common grasses (out of about 300 species) of Uttar Pradesh.

With a view to publishing a supplement to Duthie's Flora, the study of the Gramineae of the Upper Gangetic Plain was taken up by the author jointly with M. B. Raizada, Forest Botanist, Dehradun and R. C. Bharadwaja, in the year 1949.

A hunt for published work on Indian Gramineae, particularly of the Upper Gangetic Plains was started. The references collected during this search were

those pertaining chiefly to systematics and common economic uses. A large number of references, however, came to my notice which were not of immediate use for the revision of Gramineae of Upper Gangetic Plain but gave useful material for a study of grasses of India as a whole. A list of such references, therefore, which do not occur in the bibliography at the end of our Gramineae of Upper Gangetic Plain (Ind. For. Rec. N. S. 1957 : **4** (7)) was drawn and forms the chief subject matter of the present work. **This bibliography therefore, is a complementary list** to that work and for comprehensive information should be consulted jointly with that.

The study of the agricultural aspect of Gramineae has been kept alive in the various agricultural and other research institutions such as the Indian Agricultural Research Institute, New Delhi, Rice Research Station, Cuttack, Institute of Plant Industry, Indore, Sugarcane Research Institute, Coimbatore and Forest Research Institute, Dehradun, where useful work on their cultivation and economic exploitation has been done. No plant group even approaches proximity of Gramineae in economic importance. Their single use as cereal food all over the world cannot be over-emphasized. In addition to this, fodder, paper pulp, sugar, soil-binding, lawns, thatching and essential oils are some of their other important uses.

The lack of proper knowledge of the entire grass flora of our country, especially the great confusion in their identification and nomenclature, has greatly handicapped satisfactory solution of problems concerned with hybridization of cereals and sugarcane, grasslands, soil-conservation and exploitation for fodder, paper pulp, and essential oils.

Of recent the group has attracted the attention of taxonomists, morphologists, ecologists and cytologists in our country and their joint efforts will undoubtedly result in fuller knowledge of this group in India. In 1909, Blatter published the first bibliography of the Botany of British India. It included about 1500 entries. Supplementing this work, Santapau in 1952 published 'Contributions to the Bibliography of Indian Botany', which appeared in two parts, the first dealing with general and local floras, and second, dealing with monographs and papers on Families of Phanerogams. Santapau wrote in the introduction of his paper 'such bibliographies are of great help to students, but unfortunately they are all too rare in India'.

I hope that the present bibliography will be found helpful by all workers interested in the botany and applied aspects of Gramineae. To be able to write a grass flora of any region, small or large, one ought to know what has already been done on the vegetation of that area. With that consideration the more important floras on Indian Botany have been included. Numerous lists of plants and notes on botanical trips in India have been published from time to time. They are useful as materials for more comprehensive and larger floras. Most of such papers have been included by Santapau in the first part of his bibliography and it was considered unnecessary to repeat them here. However, works which were omitted there or have appeared later have been included.

Inclusion of some general reference books on taxonomy and nomenclature as well as of some books and papers dealing with Indian genera as represented in foreign countries may, perhaps, seem to be out of place here. It has been experienced that for purposes of identification, drawing keys, revision of genera, location of types, solution of nomenclatural confusion, discussion of affinities and assessment of economic value they have to be constantly referred. Several works though chiefly dealing with agricultural or economic aspects do give useful information on cultivation, varietal differences and occurence. Such works have been included.

Many of these references have been used by the author during his work and have, therefore, been checked from original works at the libraries of the Forest Research Institute, Dehradun, Indian Agricultural Research Institute, New Delhi, Publication Division, Council of Scientific and Industrial Research, New Delhi, National Botanic Gardens, Lucknow and Botanical Survey of India, Poona and Allahabad. In other cases effort has been made to check the references from reliable catalogues or bibliographies.

Some authors unfortunately send their papers, sometimes very useful ones, in less known journals which ordinarily do not have any circulation worth the name, or in such foreign journals which have a remote chance of landing on Indian soil. Their reference in some bibliography or casual presence of a reprint in some library is the only source of laying hands on them. If some such papers have been omitted, it, perhaps, could not be helped. Even otherwise some shortcomings might be revealed during practical use of this work; the author will be grateful to readers for any useful suggestions.

The first manuscript of part of this bibliography was compiled a few years ago with joint efforts of my esteemed friend R. C. Bharadwaja (died 1959). A large number of references has since been added and the manuscript modified and enlarged.

Sri M. B. Raizada, to whom I owe my gratitude for initiating my interest in this important plant group—Gramineae, helped me by allowing library facilities at the Forest Research Institute and gave many useful suggestions. I am grateful to Prof. K. N. Kaul, Dr. J. C. Sen Gupta, Dr. H. Santapau and Dr. G. S. Puri for granting me library facilities during my stay at the National Botanic Gardens, Lucknow, Botanical Survey of India, Poona and Central Botanical Laboratory, Allahabad.

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